ANNUAL REVIEW OF PLANT PHYSIOLOGY

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VOLUME I

1950

PREFACE

The launching of a new publication in these days of ever-swelling flow of scientific communication calls for a statement of purpose and definition of scope. The multiplicity of publications which now characterizes every field of scientific activity has made it extremely difficult, if not impossible, for the individual research worker, teacher, or advanced student to keep abreast, in a systematic manner, of significant developments in a major discipline, particularly in those sectors which lie outside his immediate field of specialization. The great need for integration and synthesis in a broad field of science is best served by critical reviews prepared at regular intervals by creative workers in their respective areas of activity.

Several important phases of plant physiology, such as inorganic nutrition, photosynthesis, and growth substances, have been reviewed at regular intervals in the *Annual Review of Biochemistry* ever since that publication was launched in 1932. Over the years these articles have justifiably earned for themselves a position of distinction and merit, but their continued appearance in the *Annual Review of Biochemistry* has become increasingly a subject for discussion. The ever-growing volume of activity in these and related fields of plant physiology brought in demands for greater space allocation, which the *Annual Review of Biochemistry* could not grant and still remain within the accepted limits of one annual volume. Overriding these practical difficulties was the fundamental fact that the existing reviews failed to cover the entire field of plant physiology, or even those areas in which chemical or physical approaches have been traditionally used.

In 1947 a canvass of a representative group of plant physiologists in the United States disclosed a virtual unanimity of opinion on the desirability of establishing an *Annual Review of Plant Physiology*. This expression of views served as a basis for a decision by the Board of Directors of Annual Reviews, Inc. to sponsor the new publication. It was also agreed that topics of special relevance to plant physiology, which have hitherto appeared in the *Annual Review of Biochemistry*, will henceforth be transferred to the *Annual Review of Plant Physiology*. This arrangement will not only avoid duplication and make possible more extensive coverage, but will also provide an opportunity for students in one aspect of plant physiology, such as photosynthesis or respiration, to become introduced to contemporary research in other phases of plant physiology.

The *Annual Review of Plant Physiology* will endeavor to pursue the policy established by the parent publication of stressing critical evaluation of published results of research rather than striving for encyclopedic completeness. The rotation of reviewers will ensure that fresh points of view and differences in perspective and emphasis among different workers of distinction in the same field will find expression in successive volumes. The contents of each volume are to be built around a core of annual reviews in areas of great

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activity, such as inorganic nutrition of plants, photosynthesis, and growth substances. In addition, other reviews are to be included at biennial or longer intervals, depending upon the extent of current research activity and publication. The long periods required for growing plants and for the completion of experiments in many types of investigations establish the need for less regular reviews in a volume devoted to plant physiology. An attempt will be made to seek complete coverage of the field of plant physiology over a period of several rather than a single year. Calendar regularity for individual topics will be observed only when justified by the volume of original research contributions. Within those limits the mission of the *Annual Review of Plant Physiology* is to provide annually a critical evaluation of all branches of plant physiology. Special consideration will be given to subjects bordering on several disciplines, such as soil and plant interrelations, physiological anatomy, and physiological ecology, and to the examination of the application of physiological principles to horticulture, agronomy, or forestry. It is our hope that the new publication will materially contribute to the advancement of the profession by knitting closer ties between physiologists active in agricultural specialties and those engaged in fundamental research. It is also our desire that the *Annual Review of Plant Physiology* be of distinct service in teaching at an advanced level.

We wish to express our gratitude to the Board of Directors of Annual Reviews, Inc. and its Managing Editor, Dr. J. Murray Luck, for their readiness to launch the new publication and to underwrite the attendant financial obligations. Our sincere thanks are due to the reviewers, whose contributions have created this volume, for their painstaking efforts on behalf of a publication which was yet to be born. In addition, we would like to acknowledge the loyal services of our editorial assistants and the great help given us by our printers, the George Banta Publishing Co., in the preparation of the first of this new series.

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TOPICS AND AUTHORS

ANNUAL REVIEW OF PLANT PHYSIOLOGY

VOLUME II (1951)

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MINERAL NUTRITION OF PLANTS[1]

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Occurrence of Nutrient Elements in the Soil

Nutrient elements may occur in the soil (*a*) in the aqueous solution, (*b*) adsorbed on organic or inorganic soil colloids, (*c*) in the form of an insoluble inorganic compound, and (*d*) as a constituent of organic compounds, either as a residue of plants or animals or in living organisms. The uptake of nutrients by the plant root is closely related to the form in which the elements occur. In general the availability increases in the direction $c < d < b < a$.

Potassium.—This will be mainly found in (*a*), (*b*), and (*c*), but only sporadically in organic residues. As an insoluble inorganic compound it is found in minerals. To test the release of available potassium from potassium-bearing minerals Graham & Turley (1) carried out experiments with three distinctly different types: Missouri granite, Wyomingite and glauconitic dolomite containing 5, 11.4, and 5 per cent of potassium respectively. These minerals were ground, mixed with hydrogen-clay from the heavy subsoil layer of Putnam silt loam and allowed to stand for several months. A considerable amount of potassium was released from Wyomingite, but a much smaller amount from glauconite, apparently due to the neutralizing effect of the carbonates of calcium and magnesium contained in this mineral. Practically no release was observed in the case of granite. When the mixtures of hydrogen-clay and minerals were supplied to soybeans a decided uptake of potassium was obtained in the case of glauconitic dolomite. From Wyomingite a slight potassium uptake was observed, but none from granite. Five times freezing and subsequent thawing doubled the amount of potassium released from Wyomingite, increased that coming from glauconitic dolomite, but did not affect the release from granite. These experiments demonstrate the value of certain minerals as a source of potassium for plant nutrition. In most soils potassium adsorbed by clay or humus colloids is of much more direct importance for plant nutrition than that derived directly from soil minerals.

Since in many soils the cation-adsorbing capacity depends on organic matter, the question of the availability of potassium adsorbed by humus as compared with that of clay minerals assumes much importance. Jones (2) using highly purified humic acid made a comparison of potassium adsorption by this compound with that of hydrogen-bentonite clay. When both colloids were present in equivalent amounts in the same solution, it was found that the clay had fixed about twice the quantity held by humic acid, indicating that hydrogen-bentonite had the greater affinity for potassium. This conclusion is in agreement with the results obtained in two further experi-

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[1] This review covers the period from approximately October 1948 to October 1949.

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ments. Samples of a peat and a loam containing equal exchange capacity and equal amounts of exchangeable potassium were subjected to six subsequent washings with water or carbonated water. The six washings with water extracted 11 per cent of the exchangeable potassium from the loam and 47 per cent from the peat. For carbonated water the respective figures were 16 and 69 per cent. In a second set of experiments corn was grown in quartz sand supplied with a nutrient solution complete except for potassium, which was added either in a humate or a clay (bentonite) exchangeable form. Of the 60 mg. of potassium added the corn was able to take up 40 mg. from the potassium humate and 28 mg. from the potassium-clay. The results of these experiments are in agreement with those obtained in fertilizer trials on peat and clay soils in the Netherlands. In these experiments potassium was supplied in increasing quantities. Considerably higher amounts of potassium were taken up by the plants from the humus soils than from the clay and the decrease of starch content of potatoes at high applications which was found to be due to excess of potassium was observed on the humus soils to a higher extent than on the clay soils. As a consequence of the low fixing power by humus colloids losses of soil potassium may result not only from luxury consumption by the plants but also from leaching during wet periods.

Phosphorus.—Although phosphorus is found in the aqueous solution of the soil, the greater part of this element is being adsorbed by soil colloids or fixed as insoluble inorganic compounds of calcium, iron, or aluminum. Furthermore a great deal of soil phosphates may be found in organic compounds either as such or combined with some inorganic material. The type of fixation of phosphorus in soils which is a cardinal point in phosphorus nutrition of the plants has been studied by many authors. A great number of papers deal with the use of radioactive isotopes in order to distinguish between the effects of added phosphate and that originally present in the soil. Swenson *et al.* (3) studied the mechanism of phosphorus fixation by compounds of iron and aluminum and determined the ability of certain organic and inorganic anions to prevent the fixation of phosphate by these compounds. They found that precipitation of $H_2PO_4^-$ by iron and aluminum salts to which OH^- was added took place in the form of basic iron or aluminum phosphate $Fe(H_2O)_3 (OH)_2 H_2PO_4$. This was the case even when the solution contained sufficient quantities of phosphate to occupy three of the coordination positions of iron or aluminum. Maximum fixation of phosphate was found to occur at pH 2.5 to 3.5 (iron) and at pH 3.5 to 4 (aluminum) but approximately 90 per cent of the phosphorus was still fixed by iron and aluminum at pH 6.5. To release 50 per cent of the phosphate chemically combined with iron or aluminum would necessitate raising the pH of the soil to between 7 and 8 for iron and even higher for aluminum. A similar chemical fixation of phosphate was obtained with powdered iron containing soil minerals (limonite and goethite) and with powdered kaolin (containing hydrated aluminum). Several organic anions, particularly citrate, were found to be able to prevent fixation of phosphate by iron and aluminum. These anions form complexes with iron and aluminum which are more stable than the

basic iron and aluminum phosphates. Humus and lignin and certain inorganic anions (arsenates and fluorides) were also found to be capable of preventing phosphorus from combining with iron. The beneficial effect of organic matter on availability of soil phosphate was attributed to the formation of organic acids during its decomposition (3). McAuliffe *et al.* (4) studied the reaction between phosphate and soils using radioactive P^{32}. They demonstrated that two distinct reactions are involved, one which proceeds rather rapidly and a second which proceeds more slowly. The former represents an adsorption on the surface of soil colloids. The surface-held phosphate may be considered available for plants. A more or less similar result was obtained by Barbier *et al.* (5) in a field experiment in which phosphatic fertilizers were added to a slightly acid loam for a number of years, the soil being kept free from vegetation.

Organic phosphorus in the surface layer of many soils may constitute one third and in some cases, such as prairie soils, as much as two thirds of the total phosphorus present (6); phytin, phytin derivatives and nucleoproteins apparently form the bulk of organic phosphorus compounds in the soil. Although in culture solutions these compounds are readily absorbed by the plants, their availability in soils may be considerably reduced due to fixation. Iron and aluminum salts of phythin, for instance, are less soluble than those of inorganic phosphate. Nucleoproteins may be completely adsorbed by clays of the montmorillonite type as a result of which enzymatic dephosphorylation is greatly reduced (6).

The effect of microorganisms on increase of available phosphorus in the soil may be of a different character. Gerretsen (7), employing the sterile-culture technique, has shown that the availability of phosphorus from di- and tricalcium phosphates and from ferrous phosphate was much increased by inoculating with a suspension of soil microorganisms the quartz sand to which these phosphates were added. The solubilizing effect in these experiments apparently was due to carbonic acid produced by the microorganisms living in the rhizosphere. It may be expected that this type of microbiological activity is largely confined to neutral or alkaline soils. In view of the beneficial effect of citric acid and some other organic acids on the release of phosphate fixed by iron and aluminum (3), it may be expected that organisms which produce these organic acids will increase the availability of soil phosphate. Similar results may be expected from the presence of nuclease-containing organisms with regard to the breakdown of organic phosphorus compounds of the nucleic acid type.

In order to determine what portion of the phosphorus taken up by the plants was derived from supplied fertilizers and what portion from soil phosphate, the isotope technique was employed by a number of workers (8 to 16). Dean *et al.* (8) used three soils with a different phosphorus status. It appeared that perennial rye grass growing on a soil poor in phosphorus derived nearly 80 per cent of its absorbed phosphorus from applied superphosphate. For the moderately poor and the rich soils these values amounted to 60 and 10 per cent respectively. In a second study (9) utilization of fer-

tilizer phosphorus by different crops was studied. In potatoes over 50 per cent of the absorbed phosphorus was derived from fertilizer phosphate throughout the growing season. In corn, values of 60 per cent (early growing period), 25 per cent (second harvest) and 10 per cent (third harvest) were found. Potatoes responded consistently to the phosphatic fertilizers, whereas corn responded only in the first growing stage. Similar results were obtained by Krantz *et al.* (10) in field trials with corn, cotton, potatoes, and soybeans. Potatoes, although having absorbed considerably lesser amounts of total phosphorus than soybean and corn, had taken up much more of the fertilizer phosphorus. Jacob *et al.* (11), in field experiments with potatoes, found that the level of soil phosphorus did not greatly affect the amounts of phosphorus absorbed from fertilizers. With increased soil phosphate content considerably more soil phosphorus was taken up, however, as a result of which the percentage of phosphorus in the plant, derived from fertilizer, decreased as the available soil phosphorus increased. Similar results were obtained by Woltz *et al.* (12) in field experiments with tobacco. In the experiments of Jacob *et al.* (11) it was demonstrated that soil reserves in the presence of phosphatic fertilizers supplied less phosphate to the plants than they did in the absence of these fertilizers. This result is in agreement with that obtained by Barbier *et al.* (5) in experiments with separated root systems.—

The percentage of phosphorus in the plant derived from fertilizer phosphorus depends upon the type of fertilizer used [Blaser & McAuliffe (13)]. With superphosphate considerably higher values were obtained than with dicalcium phosphate, tricalcium phosphate and calcium metaphosphate. Similar results were obtained by Hall *et al.* (14) with cotton and corn, by Stanford & Nelson (15) with oats and alfalfa, and by Olsen & Gardner (16) with sugar beets, wheat, and barley. McAuliffe *et al.* (17) investigated the uptake by Italian rye grass of P^{32}, incorporated in stable manure and in superphosphate. Only 16 per cent of total phosphorus was present in organic form in the manure, mainly protein-bound. More phosphorus was taken up from superphosphate than from manure when both materials were mixed with the soil. The utilization of protein-bound phosphorus was only 20 to 30 per cent of the value obtained with superphosphate. White *et al.* (18) studied the utilization of phosphorus incorporated in green manure crops by the succeeding crop. P^{32} was supplied to alfalfa plants, the tops of which were supplied as phosphorus source to sudan grass. For comparison P^{32} was supplied as KH_2PO_4. It appeared that on a very deficient soil, the green manure and KH_2PO_4 were equally effective as sources of phosphorus. Fuller & Dean (19) carried out similar experiments under greenhouse conditions. Soybean and wheat, grown on a soil treated with P^{32}, were used later as green manure. Since these plants contained a considerable portion of their phosphorus in the inorganic state, parts of them were subjected to a treatment in order to remove the inorganic phosphorous compounds. Rye grass absorbed the phosphorus from the untreated green manure about 70 per cent as efficiently as it did from the superphosphate. Uptake from organic phosphate was much less, however.

Manganese.—Of the micronutrient elements manganese, in particular, has been investigated by many soil chemists. Although the element may occur in relatively large quantities in most soils, it may be completely unavailable for the plant when certain circumstances prevail in the soil. Earlier investigations have shown that these involve the presence of a certain amount of organic matter and the soil reaction being weakly acid to weakly alkaline. It is believed that the conversion of available manganous compounds to unavailable MnO_2 is brought about by soil microorganisms [Gerretsen (20), Leeper & Swaby (21), Quastel *et al.* (22)]. Other investigators (24) are of the opinion that the fixation of manganese is not a result of microbiological activity because of the great rapidity with which this process takes place. According to Gerretsen (20) the microbiological conversion of manganous compounds proceeds between pH 6.3 and 7.8 with an optimum at 7. Leeper & Swaby (21) found much wider pH limits viz., 5.5 to 8.5. Reduction of higher manganese oxides may result as a direct reaction with organic matter, particularly at low pH values, since the oxidizing power increases rapidly with acidity. Microbiological reduction can take place at any pH value if the oxygen tension is low. Quastel *et al.* (22) reported beneficial effects on reduction of higher oxides of manganese in soils of such reducing agents as glucose, hydroquinone, thiols, polyphenols, and thiosulphates. In experiments using the soil-perfusion technique an increase of divalent manganese, after addition of sulphur, was found before there was any appreciable change in pH. It was suggested that this effect was due to the formation of thiosulphate. In pot experiments with oats and beets the beneficial effect of thiosulphate appeared to be transient.

According to Leeper (23) MnO_2 in different soils may react in a different manner with reducing substances. Highly reactive MnO_2 may be reduced by quinol within 15 sec. A second type may react with hyposulfite at pH 7 but not with quinol, and inert MnO_2 does not react with either of these solutions.

Fujimoto & Sherman (24) studied the effect of a number of treatments on availability of manganese in Hawaiian soils. Most of the chemical treatments investigated in the laboratory were duplicated by pot experiments with cowpeas. A light application of calcium carbonate reduced the available manganese tremendously. With increased quantities of calcium carbonate manganese absorption was gradually reduced. Sulphur, sucrose, ground pineapple, and sugarcane leaves with a high carbon-nitrogen ratio gave a considerable increase in available manganese. When the soils were ovendried a considerable increase in available manganese was obtained. According to Fujimoto & Sherman (24) a certain portion of the manganese in the soil exists as a complex $(MnO)_x (MnO_2)_y (H_2O)_z$. When the water of hydration is split off (drying at relatively high temperature), the rest of the complex becomes unstable and breaks up into its component parts, MnO plus MnO_2.

An increase of exchangeable manganese after treatment of the soil with organic materials with a carbon-nitrogen ratio of 30:1 was also observed

by Hurwitz (25). This was particularly the case at a temperature of 37°
and 47°C. At 30° or lower only a slight rise was found. The increase in avail-
able manganese was attributed to a much enhanced growth of microorgan-
isms as a result of which oxygen was removed and reduction of higher
oxides of manganese took place. Upon steam sterilization of soils an enormous
rise in exchangeable manganese was found (26). Owing to this, plants grow-
ing afterwards in these soils contained high amounts of manganese as a
result of which injury occurred.

Heintze & Mann (27) have shown that organic soils may fix divalent
manganese in forms not readily exchangeable with ammonium acetate. In
the recovery of this manganese, copper-ammonium complex ions were found
to be particularly effective. The result here was complicated by the fact that
copper can act as a catalyst in the oxidation of soil organic matter and the
reduction of higher oxides of manganese in soils. The hypothesis was ad-
vanced by these authors that manganese deficiency of plants occurring on
neutral and alkaline soils of high organic matter content and of adequate
total manganese content is due to the formation of complexes of divalent
manganese with the organic matter which are dissociated only to such a
slight extent that available manganese in the soil solution is insufficient for
the needs of the plants.

Copper.—Although the copper content of most soils is very low, defi-
ciency symptoms of the plants as a result of an absolute lack of copper are
found only sporadically. Presence of certain types of peaty substances will
often reduce the availability of soil copper, as a result of which deficiency
symptoms in the plants occur. In his earlier work the writer has shown (28)
that such substances are able to fix relatively large amounts of copper in
such a way that it was unavailable for *Aspergillus niger.* Furthermore it was
demonstrated that hydrogen-sulphide producing bacteria are able to fix
copper in an unavailable form. This was not simply copper sulphide, since
copper supplied in this form was found to be readily available for plants and
microorganisms like *A. niger.* In contrast to the results of these experiments
and those of Steenbjerg (29), Lundblad *et al.* (30) came to the conclusion
that copper deficiency of plants in Swedish soils is due to the absence of
copper from these soils and not to the presence of copper-fixing organic
matter. The behavior of copper in organic soils is different from that of
other cations. Lucas (31) observed that copper sulphate was precipitated,
probably as the hydroxide, when the pH of the soil-water suspension was
greater than 4.7. When a hydrogen-soil was treated with copper acetate,
copper was adsorbed as the divalent cation Cu^{++} and as the monovalent cat-
ion complex $(CuCH_3COO)^+$. Hurwitz (32) found that addition of oat straw
and alfalfa meal with a carbon-nitrogen ratio of 30:1 gave a rise in exchange-
able copper of a sandy loam from 8.4 to 22.0 μg. per 100 gm. of dry soil.
This increase was not due to the presence of copper in the organic matter.
When the amended soil was incubated at 29°, 37°, and 45°C. for 14 days,
available copper had again reached the initial low level. At 2° no change was

found, indicating that the organic compounds which were responsible for the increase of exchangeable copper were decomposed by microbiological activity.

INTAKE OF MINERAL NUTRIENTS

Intake of mineral nutrients by the plant depends partly on the composition of the nutrient medium (soil), partly on the type and condition of the roots. As to the former, the following points may be mentioned: (*a*) amount and type of soil colloid (absence or presence of a crystal lattice in which cation fixation in difficult or nonexchangeable form may take place): the higher the exchange capacity of a soil colloid the easier will be the release of monovalent cations in comparison with that of divalent ions; this was shown by Elgabaly & Wiklander (33, 34) in experiments with barley and pea plants using kaolin and bentonite with a base-exchange capacity of 3.0 and 90 m.eq. per 100 gm. respectively; at equal concentrations and ratios of adsorbed sodium and calcium the plants absorbed relatively more calcium and less sodium from kaolin than from bentonite; (*b*) concentration of exchangeable ion on the colloid: as the concentration decreases the exchangeability (availability) will become more difficult; (*c*) ease of release of the ion as expressed by the activity coefficient of the ion in the adsorbed condition, and (*d*) relative concentration and type of other ions (complementary ions) on the colloid. Exchangeability of an adsorbed ion increases when a complementary ion with a high activity is replaced by an ion of low activity (activity coefficients: $Li > Na > K > Mg > Ca > Sr > Ba > La$). Wiklander & Gieseking (35) studied the effect of type of complementary ion on the exchangeability of adsorbed cations. Amberlite IR-1 was used as "ideal exchanger" because it shows no crystal lattice type of cation fixation as is the case with clay and other natural exchangers. Hydrogen-amberlite was neutralized with the following pairs of ion combinations: potassium-sodium, potassium-barium, strontium-sodium, strontium-barium. The proportion of each ion in each pair was varied over a wide range including very low values. It was found that with falling potassium saturation the exchangeability of potassium decreased when combined with sodium but increased when combined with barium. Strontium showed similar relationships to that of potassium. The importance of complementary ion effect on uptake of plant nutrients was also shown by Mehlich & Reed (36). With increasing magnesium and potassium levels in the soil, release of calcium generally decreased (concluded from chemical tests with dilute hydrochloric acid and absorption experiments with soybeans, oats and turnips). Influence of type of root on absorption of cations was shown by Elgabaly & Wiklander (34, 37). In agreement with their view that Donnan equilibrium phenomena determine the uptake of ions, it was shown that roots with a low exchange capacity (acidoid content) like barley absorbed more monovalent than divalent cations, whereas pea roots with a high exchange capacity behaved reversely.

Lundegårdh (38) has extended his theory on anion uptake and aerobic

respiration of roots of wheat. Earlier investigations of this author have shown that uptake of anions by roots is linked with part of the aerobic respiration, the salt or anion respiration. This fraction of the respiration is very sensitive to cyanide and is probably identical to the cytochrome-cytochrome oxidase system. The remaining fraction, insensitive to cyanide, (ground respiration), is not related to anion absorption. As to the anion respiration it is suggested that one monovalent anion will be transported for each electron operating in the cytochrome-cytochrome oxidase system. If this hypothesis is valid, the quotient $q(an./O_2)$ = equivalent absorbed anions/molecules consumed oxygen, should have a constant value. Since 24 steps of electron transfer are concerned in complete oxidation of glucose, $q(an./O_2)$ should have a value of four. This theoretical value was approached in experiments with slices of storage tissue by Robertson & Wilkens [cited in ref. (39)]. In wheat roots much lower values were obtained, and changes were found when conditions were changed. Lundegårdh (38) studied a number of factors which may be responsible for these discrepancies: (*a*) bleeding of the cut wheat roots, as a result of which mineral anions are exuded in the solution and too low values for uptake will be found; by preventing the exuded sap from flowing back to the salt solution, this difficulty may be eliminated; (*b*) anion respiration is not only linked with absorption of anions from the nutrient solution but also with internal transport of mineral and organic anions; (*c*) in experiments with different zones of wheat roots it was found that the tip zone (0 to 30 mm.) is provided with a third type of aerobic respiration which is missing in the zone 30 to 60 mm. This "third" respiration was found to be characterized by a moderate sensitivity to cyanide in contrast to the anion respiration which is highly sensitive and the ground respiration which is insensitive. In some sets of experiments in which bleeding errors were avoided $q(an./O_2)$ values were determined at different cyanide concentrations. In contrast to the experiments of Robertson & Wilkens (39) with storage tissue in which q values of nearly four were obtained, a quotient of one was found in wheat roots (38). Decrease of anion absorption with increased cyanide concentration closely followed decrease of oxygen consumption so that constant q values were obtained. A value of one means that only one fourth of the anion respiration can be accounted for by anion absorption.

In a second paper (40) Lundegårdh compared growth, bleeding, and salt-absorption phenomena in wheat roots. It was shown that the bleeding of detached roots which represents the ascending sap stream runs fairly independently of the salt accumulation process (it continues in the absence of oxygen or under the influence of cyanide). It is indirectly dependent on the salt accumulation, however, since the latter furnishes the roots with salts. It was found that bleeding is linked to glycolytical processes (inhibitory effect of small quantities of iodoacetic acid and fluoride). Although growth is generally linked to aerobic respiration, no close relation was found to exist between ion absorption and growth.

Woodford & Gregory (41) have studied the relation between salt absorp-

tion and respiration in barley roots. In contrast to Lundegårdh (38, 40) and Hoagland (42) they used whole plants. At all oxygen levels (0, 20, 40 to 100 per cent), absorption of nitrate was found to increase with the concentration of this ion, but the slope of the curves was steeper at a higher oxygen level. The fact that uptake of nitrate was found to take place in the absence of oxygen in the nutrient solution does not necessarily mean that this process takes place in the absence of oxygen, since the roots may have been supplied with oxygen from the aerial parts. Removal of the shoots caused an immediate fall in nitrate uptake, but did not affect respiration until several hours after excision, after which both respiration and uptake decreased. In a further experiment uptake of potassium, nitrate, and phosphate and respiration of intact roots were determined at four oxygen levels in combination with four nutrient levels. Respiration increased considerably from the first to the second nutrient level. With further increase in nutrient supply, respiration remained approximately constant notwithstanding that the uptake of salts increased markedly. Over the main range of uptake the respiration rate, therefore, was found to be independent of the nutrient absorption and was determined merely by the oxygen tension in the culture solution. Absorption of nitrate and potassium increased throughout the range of oxygen tensions with the greatest slope between 0 and 5 per cent oxygen. In the case of phosphate uptake, however, the values decreased considerably at 5 per cent oxygen. With a higher oxygen supply phosphate uptake was higher again but it never reached the values obtained in pure nitrogen. Although the results of these experiments differ widely from those obtained by Lundegårdh, it should be stressed that the latter used excised roots in short-term experiments whereas Woodford & Gregory did their main experiments with whole plants. The duration of the experimental period was considerably longer in these tests.

Rees (43) in experiments with slices of storage tissue observed a favorable effect of prolonged washings in aerated running tap water on subsequent uptake of both manganese and chloride from a solution of manganese chloride. Alberda (44) investigated the uptake of phosphate by intact plants of *Zea Mays* using low-phosphate and high-phosphate plants. The former absorbed phosphorus at a rate twice as high as did the high-salt plants. This difference was maintained for a number of days. The uptake of phosphorus was found to run parallel to the growth of the whole plant. Illumination was shown to be a primary factor in phosphorus absorption, apparently owing to reduced carbohydrate supply in the dark. Absorption of phosphorus was found to be independent of the concentration of other anions (nitrate, sulphate, chloride) in the nutrient solution. As opposed to the latter results Mattson *et al.* (45, 46) observed a clear effect of neutral salts on phosphorus uptake by pea and barley from very dilute solutions. This result is in accordance with their view that Donnan equilibrium phenomena determine the rate of uptake of anions and cations by plant roots. In agreement with the Donnan theory calcium chloride was found to have a greater stimulating

effect than potassium chloride. Pea roots were found to absorb phosphate much more slowly than rye roots. The former had a very high cation exchange capacity, 71 m. eq. per 100 gm. of dry matter against 29.5 m. eq. in rye and 25.3 m. eq. in barley roots. It was suggested that the high acidoid content is due to the occurrence of pectine in the surface layer of the roots.

PHYSIOLOGICAL FUNCTIONS OF NUTRIENT ELEMENTS IN THE PLANT

Potassium.—Although at one time or another almost every important physiological process in the plant has been ascribed to potassium, the mechanism of its role in any specific process has not been elucidated [Hoagland (42)]. Cooil (47) in experiments with guayule found a higher content of citric acid in plants supplied with large amounts of potassium than in those with a low or moderate supply of this element. Malic acid was found to be high in potassium-deficient leaves and decreased considerably with increased potassium supply. These results are in agreement with the findings of Juul (48) that potatoes fertilized amply with potassium contain considerably more citric acid than those poor in potash. In agreement with the results of earlier investigations Cooil & Slattery (49) noted a higher content of carbohydrates, particularly reducing sugars, as well as of nitrogenous compounds (amide and amino nitrogen) in the leaves of potassium-deficient guayule. Similar results were obtained by the reviewer in fertilizer experiments on pastures (50). Since in these (49) as well as in earlier investigations the ratio soluble organic nitrogen/protein nitrogen was higher in potassium-deficient plants than in normal ones, the suggestion was made earlier that potassium plays a part in the synthesis of protein from soluble compounds. More evidence is needed, however, to prove the validity of this hypothesis.

The finding of Cooil & Slattery (49) that an almost linear relation exists between the ratio levuline plus inulin:reducing sugars and the ratio potassium:soluble calcium indicates that potassium plays a part in condensation processes. Mulder (51) studied the effect of potassium on tyrosine content and tyrosinase activity of potato tubers. Of the five elements tested, nitrogen, phosphorus, potassium, magnesium and copper, potassium had by far the greatest influence on the free-tyrosine content of the tubers. Much higher values were found in potassium-deficient tubers than in normal ones. Tyrosine content of tuber protein appeared to be unaffected by the potassium supply of the plants. Tyrosinase activity of tuber tissue was found to be independent of the potassium nutrition. The blackening of potassium-deficient potatoes induced by bruising was shown to be due to the enzymatic oxidation of free tyrosine and of an *o*-diphenol also present in relatively large amounts in these tubers, first to a red pigment and then to the bluish-black melanin. In normal tubers this reaction does not take place because of the localization of enzyme and substrate in different parts of the cell and the presence of a reduction system. Disturbance of the cell structure following injury to the tissue will allow the tyrosine oxidation to proceed. Potassium-deficient tubers and particularly their stem ends appeared to be much more liable to injury than tubers with a normal potassium supply.

Potassium-sodium relation.—Lehr (52) has studied the effect of sodium nitrate as compared with that of calcium nitrate on growth and cation uptake of spinach growing on soil and on artificial soil mixture at three different potassium levels. Much higher yields of the sodium-treated plants were obtained, particularly at a low potassium level, so that Lehr assumed that sodium may replace potassium to a large extent. The fact, however, that at the highest potassium level sodium nitrate gave still higher yields than calcium nitrate, makes it highly probable that sodium exerts some specific effect in spinach. Whether this effect is of a secondary character, e.g., preventing toxic accumulation of calcium as Richards assumes (53), or is owing to some specific physiological function in the plant has to be left undecided. Large amounts of sodium were absorbed by the spinach plants at low potassium levels. With an increased potassium supply the sodium contents were much reduced showing that spinach plants have a certain preference for potassium.

Phosphorus.—A great deal of the investigation concerning phosphorus in plant development is based on the isotopic tracer technique. Although it is generally assumed that radiation damage to the living organisms in tracer experiments is negligible at the concentrations used, Russell & Martin (54) have reported that this may not be the case. Young barley plants were treated with P^{32} varying from 0.5 to 50 microcuries per l. of culture solution over periods of four to six days. Considerable reduction in root yields and in phosphorus absorption was obtained after six days' treatment with P^{32}. This effect was greatest at a low phosphorus level (0.01 m. eq. per l.) at which significant reduction occurred when the dosage reached 10 microcuries per liter. Since similar values are often used in tracer investigations, the plants of any such investigation should be very carefully examined in order to be sure that no changes occur as a result of radiation. On the other hand, when radiation damage is found, special consideration should be given to the possible presence of toxic contaminants in the radioactive material. No damage from applying P^{32} at 26 and 260 microcuries per gm. of phosphorus to wheat under field conditions was observed by Dion *et al.* (55). No data for yield of roots and phosphorus absorption were reported, however. According to Russell & Martin (54) both these values are more affected by radiation damage than the yields of tops. Miller & Wolken (56) investigated the effect of radioactive phosphorus on the growth of two species of fungi and their decay in oranges. Retardation in growth was observed in nutrient media containing P^{32} in a concentration of 3.1 to 4.9 microcuries per liter. Although no radioactivity could be detected in the inoculum at the time that the oranges had been inoculated, infection of the fruits was markedly reduced in the case of P^{32}-treated fungi.

A study on translocation of phosphorus in maize based on the isotope technique and using the root separation method was made by Moore (57). It was found that the average upward rate of flow of P^{32} in the shoot was 4.2 cm. per hr., the downward rate of flow in the root not supplied with P^{32} averaged 4.0 cm. per hr. Labelled phosphorus in no case appeared in the

lateral roots before it was distributed throughout the shoot, indicating that the downward movement into the roots is not a simple diffusion. Four days after supplying P^{32} to one half of the nutrient solution it was detected in the other half. Downward movement was greater when the roots were growing in a phosphorus-deficient medium than in a solution supplied with phosphate.

Van der Paauw (58) has studied the formation of organic matter, the absorption of phosphorus and nitrogen, and the distribution of these substances in potato plants grown under field conditions at four different phosphorus levels. In agreement with the results obtained by van de Sande Bakhuyzen (110) in experiments with wheat, a constant distribution of newly formed organic matter among leaves, stems and tubers was found to exist for considerable lengths of time. At certain physiological stages in the development of the plant, sudden changes in this distribution occurred. A first change was found to take place at the tuber initiating stage, a second when the weight of leaf began to decrease. With regard to uptake of phosphate and nitrogen similar relations were observed. Absorption of nitrogen stopped as soon as the leaves had reached their maximal development, the absorption of phosphorus however, continued until the end of tuber growth. Apparent assimilation per unit leaf weight was found to be higher in plants supplied amply with phosphate than in those with inadequate phosphate nutrition.

In agreement with the important part played by phosphorus in the energy metabolism of living organisms, Wassink *et al.* (59), in experiments with purple sulphur bacteria, have provided evidence that these photo-autotrophic organisms in the light and in the presence of hydrogen but in the absence of carbon dioxide, are capable of building up energy-rich phosphate bonds. This was concluded from the fact that phosphorus disappeared from the solution when a suspension of *Chromatium*, aerated with a mixture of oxygen-free nitrogen and hydrogen was exposed to light. Shift to darkness resulted in a marked release of phosphate, apparently as a result of breakdown of the energy-rich phosphate bonds.

Calcium.—Calcium is an important element in root development and root functioning. This was shown by the experiments of Haynes & Robbins (60) who cultivated tomato plants in dual cultures so that different nutrient treatments could be applied to different portions of the root system. When one half of the roots was given a complete nutrient solution, except calcium and boron and the other half calcium plus boron, the roots in the former medium died, whereas in the latter they made a healthy appearance. Similar poor results were obtained when either calcium or boron was left out of the nutrient medium. In this case the growth of the roots was abnormal, and deficiencies of the major nutrients were seen in the tops. Apparently the presence of both calcium and boron in the nutrient medium is essential to the growth and functional integrity of the roots. Presley & Leonard (61) demonstrated the importance of calcium in the development of the radicle of cotton seedlings. Although earlier investigations have shown a beneficial

effect of boron on calcium uptake in maize, broad bean, and soybean, no such effect was found by Brennan & Shive (62) in tomato.

The experiments of Brady (63) and Harris (64) have shown that calcium plays an important role in the fruit formation of peanut. These fruits develop at the end of gynosphores or "pegs." Both fruits and pegs possess roots and may absorb nutrients. By using a special technique it was possible to apply different nutrient solutions to the main root system and that of the pegs. When calcium had been left out of the nutrient medium of the pegs the formation of normal fruits was very poor. This was true notwithstanding that the supply of calcium to the roots was normal, which indicated an inadequate translocation of calcium in peanut plants. Bledsoe *et al.* (65) came to a a similar conclusion in experiments with radioactive calcium (Ca⁴⁶). Vlamis (66) obtained symptoms of calcium deficiency in lettuce and barley when the calcium saturation of the soil colloid was below 20 per cent. This was the case when magnesium was the predominant complementary ion. When potassium was the complementary ion, an even higher calcium saturation was required to ensure an adequate absorption of this element.

Jensen (67) has investigated the effect of calcium on nitrogen fixation by *Azotobacter indicum*, an *Azotobacter* species which is able to develop and to fix nitrogen at pH values ranging from three to nine. In contrast to *A. chroococcum*, calcium was found to be unessential for *A. indicum*. Shew (68) has demonstrated a beneficial effect of calcium on development of bacteriophages of lactic streptococci.

Iron.—Earlier investigations have made it probable that iron has something to do with the blackening after cooking of potato tubers (69). Juul (48) made an extensive study of this phenomenon and came to the conclusion that the ferrous ions of the potato combine, upon cooking with an *o*-dihydric phenol, presumably caffeic acid, to give a colorless compound, which is oxidized to the strongly colored ferric compound on being exposed to air. Since the color intensity of ferric compounds of *o*-dihydric phenols increases when the pH becomes higher, it was assumed by Juul that the discoloration of cooked potatoes depends to a large extent up on the pH of the tuber tissue. Factors which promote the blackening, particularly the nitrogen: potassium ratio, were found to increase both the pH of the tuber tissue and its *o*-diphenol content. The writer, in studying blackening phenomena in raw and cooked potatoes (51) came to the conclusion that formation of melanin, which is the cause of discoloration of raw tubers, is responsible for blackening after cooking only in those cases in which red or black oxidation products of tyrosine or *o*-diphenol were present before boiling. In those cases in which the raw tubers were uncolored, blackening after boiling was found to be due to nonenzymatic processes. In agreement with the results obtained by Juul, it was shown to be caused by the ferric compound of an *o*-dihydric phenol. Potassium-deficient tubers, which are particularly prone to blackening, were found to have a much higher content of *o*-dihydric phenols than

those supplied amply with potassium. No difference in iron, soluble in dilute acetic acid, between normal and potassium-deficient tubers was found. Besides o-diphenol and iron a third factor helps to determine the degree of blackening of cooked tubers. Unlike Juul the writer is of the opinion that this factor cannot be the pH, since the slight differences in pH which were found in tissues of different potassium nutrition were unable to produce any appreciable differences in discoloration. It was suggested that the content of citric acid is of much more importance than the pH. Addition of citrate to slices of such tubers markedly reduced the intensity of discoloration. Potassium-deficient potatoes contain considerably less citric acid than those with an ample potassium supply (48).

Manganese.—There are two main problems to consider in the manganese nutrition of plants: the relation (antagonism) between manganese and other elements, particularly iron, and the function of manganese in nitrate reduction and photosynthesis. Several investigators have emphasized the importance of a certain iron-manganese ratio within the plant. Somers & Shive (70) stated that for soybean the ratio of soluble iron to soluble manganese in the plant should lie between 1.5 and 2.5. If the ratio were above 2.5 symptoms of manganese deficiency (=iron toxicity) should occur; if it were below 1.5 the plant should suffer from iron deficiency (=manganese toxicity). Morris & Pierre (71) and Berger & Gerloff (72) were unable to confirm these results as to the similarity of iron deficiency and manganese toxicity. The former grew *Lespedeza* in nutrient solutions at different manganese and iron levels. Manganese toxicity symptoms were found to be much less pronounced at a high iron level than at low concentrations indicating an antagonism between iron and manganese. The alleviation of manganese toxicity by the higher iron supply was found to be due to an approximately 50 per cent reduction in the manganese content of the plants rather than to an increase of total iron in the plant. In a further set of experiments (73) *Lespedeza*, soybean, cowpea, and peanut were grown at different concentrations of manganese in the nutrient solution. Symptoms of manganese toxicity were found to be entirely different from those of iron deficiency. Similar results were obtained by Berger & Gerloff (72) in experiments with potato. Excess of manganese produced symptoms of injury which were identical with those of stem streak necrosis occurring on acid soils. Introduction of more iron was found to be unsuccessful in preventing these symptoms. According to Sideris & Young (74) pineapple plants grown in highly manganiferrous soils became chlorotic, which was prevented by the application of sprays of ferrous sulphate solutions. They suggested that the chlorosis is due to the substitution of manganese for iron in the porphyrin compound, thereby inactivating the latter for subsequent conversion to chlorophyll. No experimental data were presented, however, to sustain this hypothesis. Gerretsen (75) in studying the effect of manganese and iron on oxidation reduction potentials in suspensions of chloroplasts observed an opposite effect of these elements on E_h upon illumination of the suspensions. Hewitt

(76) obtained iron-deficiency symptoms in beets upon treatment with several heavy elements, including manganese. Symptoms of iron deficiency and manganese toxicity were clearly distinguishable, however.

Acid soils contain a high concentration of soluble manganese. Plants growing on these soils may absorb high amounts of manganese which may cause symptoms of injury. When such soils are limed the absorption of manganese is considerably reduced (72). Apparently this is due entirely to an insolubilization of manganese and not to an antagonism between calcium and manganese, a conclusion based on the fact that calcium sulphate had no effect on manganese toxicity of potato plants. Morris & Pierre (71) in culture-solution experiments with *Lespedeza* likewise failed to show any alleviation of the manganese-toxicity symptoms with additional calcium.

A boron-manganese relationship was described by Gisiger & Hasler (77) in oats. The addition of small amounts of boric acid aggravated manganese-deficiency symptoms to a high degree, whereas large amounts gave healthy plants. [Earlier investigations by Burström (78) have shown that additions of small amounts of manganese considerably increase the rate of nitrate assimilation in excised roots of wheat plants or in macerated roots.] The effect of manganese and oxygen on nitrate reduction has been studied recently by Nance (79). In order to eliminate the well-known influence of oxygen on absorption of nitrates, roots were used which previously had accumulated nitrate. In aerated solutions the assimilation was considerably less than in those treated with nitrogen. The addition of manganese to the culture solution inhibited nitrate reduction when the roots were aerated. In nitrogen the inhibition by manganese was less pronounced. The failure to demonstrate a stimulation of nitrate reduction by manganese was ascribed to a high content of this element in the seed. No manganese determinations had been carried out, however, so that this assumption has not been verified. In the case of macerated roots the results were in accordance with those of Burström as far as the stimulation of nitrate reduction by oxygen was concerned.

Jones *et al.* (80) in culture-solution experiments with soybeans, in which the entry of oxygen to the nitrate-containing solution was prevented, observed an accumulation of nitrite in the nutrient solution when no manganese was added. The plants showed yellow leaves apparently owing to nitrogen deficiency. Supplied with small amounts of manganese, no nitrite accumulated and the plants were green and healthy. These results indicate that manganese acts as a catalyst in nitrate assimilation, particularly in the nitrite reduction step. Some investigators have found a high concentration of nitrate in the leaves and stems of manganese-deficient plants [Leeper (81), Hewitt *et al.* (82)]. In their opinion this accumulation is indicative of an essential function of manganese in nitrate reduction. However, accumulation of nitrate in chlorotic leaves may be due to a lack of carbohydrates as a result of poor carbon dioxide assimilation. No record is made of the extent of chlorosis by the authors mentioned above. Hewitt *et al.* found nitrate accumulation in both manganese-deficient and molybdenum-deficient cauliflowers. Molyb-

denum deficiency was found to result in a marked reduction in the concentration of most amino acids; manganese deficiency, however, was associated with an increased amino-acid concentration. If manganese has to be considered as a catalyst in nitrate reduction, it is difficult to see why accumulation of nitrate, nitrite (80), as well as amino acids, occurs.

The effect of manganese on photosynthesis was investigated by Portsmouth (83) and by Gerretsen (84). The former determined the increase of dry weight of normal and manganese-deficient potato leaves during the day and found almost 30 per cent higher values for apparent assimilation in manganese-deficient plants. It was suggested that these high values were due to an increased respiration or an increase in translocation in the manganese-treated plants. No experimental evidence was presented, however, supporting this hypothesis. Gerretsen (84) determined carbon-dioxide uptake of normal and manganese-deficient oat leaves and demonstrated that in the latter carbon assimilation was reduced to values as low as one third of those of the controls. In order to avoid the possibility that the low values were simply due to chlorosis of manganese-deficient plants, special care was taken to compare leaves which differed only slightly in chlorophyll content. Several symptoms of manganese deficiency of oats were traced back to shortage of carbon assimilation in the leaves. In a second paper Gerretsen (75) has described the effect of manganese on oxidation reduction potentials of illuminated crude chloroplast suspensions. When such suspensions made from normal crushed oat leaves were illuminated, a considerable rise of E_h was observed which changed into a rapid fall when the light was switched off. When during illumination oxygen was exhausted, a sharp drop in E_h occurred, but this was reversed on aeration. After the addition of small amounts of manganese sulphate to the suspensions in the dark, no change in potential resulted, but when the suspensions were illuminated E_h rose to values of 500 mv. or more. This value was 150 mv. higher than those attained without the addition of manganese. In the case of manganese-deficient leaves the rise in potential was insignificant upon illumination, but addition of a trace of manganese sulphate to the suspension, in the dark, resulted in a rise of 150 mv. when illuminated. From these results Gerretsen concluded that manganese plays a specific role in hydrogenation of oxygen to hydrogen peroxide which is responsible for the high potentials. It was assumed that the hydrogen atoms are derived from the photochemical splitting of water ($H_2O \rightarrow H$ atom $+ OH$ radical). It was suggested that manganese plays an active role in this reaction by combining with hydroxyl radicals and thus preventing the back reaction $H + OH \rightarrow H_2O$. Addition of ferric ions to the chloroplast suspension gave rise to a lowering of E_h upon illumination and a rise after the light was switched off. It was assumed that iron, eventually incorporated in an organic complex, combined with hydrogen atoms, derived from the splitting of water, temporarily stabilizing them. The hypothesis that manganese and iron are to be considered as complementary oxidation-reduction catalysts is in agreement with the results of those authors who

observed an interdependency of iron and manganese in normal plants.

Copper.—From a physiological point of view copper is an important element as a constituent of some enzymes (e.g. polyphenoloxidase = tyrosinase). It is claimed that tyrosinase plays a part as a terminal oxidase in the respiration of plants (85). Arnon (86) studying the polyphenoloxidase of *Beta vulgaris* has shown that in the leaves this enzyme is located in the chloroplasts. The cytoplasm did not show any appreciable activity. The writer (51) has studied the tyrosinase activity of potato tubers in relation to enzymatic blackening. It has been found that plants grown on soils poor in copper, although making an entirely normal appearance had a tyrosinase activity less than 1/10 that of tubers with a normal copper supply. As a result of the low tyrosinase activity, blackening of bruised potatoes deficient in both potassium and copper was slight in comparison with that of tubers deficient in potassium but supplied normally with copper.

Boron.—A beneficial effect of boron on calcium uptake as was reported earlier by Minarik & Shive (87) was observed by Henderson & Veal (88) in blue lupine. A beneficial effect of both boron and calcium on root development and function has been noted by Haynes & Robbins (60). That boron is essential not only for the development of the roots but also for the formation of root nodules of leguminous plants was shown in this laboratory (89) in culture-solution experiments with peas [see also the earlier investigations of Brenchley & Thornton (90) with beans]. In the absence of boron only rudimentary nodules were formed which were unable to fix nitrogen. In culture-solutions this happened only at very low boron levels. Supplied with small amounts of boric acid normal nodules developed but boron deficiency symptoms were seen in the shoot soon afterwards. On soils poor in boron an inadequate nitrogen fixation was observed in well-developed plants which were free from typical boron-deficiency symptoms in the shoot.

Mac Vicar & Burris (91) in experiments with tomato, soybeans, tobacco, and cabbage have shown that the uptake of oxygen by ground leaf tissue from boron-deficient leaves was considerably higher than in the case of normal leaves. Similar results were obtained by using cell-free chloroplast suspensions. Addition of boric acid to these preparations reduced the rate of oxygen consumption of boron-deficient samples, but the level of normal leaves was not attained even when boric acid was added in a concentration as high as 0.1 M. By adding various substrates to cell-free extracts of tomato tissue, it was shown that polyphenoloxydase (tyrosinase) activity was markedly higher in boron-deficient leaves than in normal ones. Reduction of oxygen uptake by a 0.001 M boric-acid solution was much stronger in both extracts supplied with 3, 4-dihydroxyphenylalanine than in the untreated extracts.

Sykes & Reed (92) demonstrated that small amounts of boric acid prevented the swarming of *Proteus vulgaris* on an agar nutrient medium. Growth of this and of other bacteria was not affected by the treatment. Electron micrographs of these organisms showed few flagella, most of which were

broken and detached. On the surface of these flagella numerous small spherical particles and agglomerations of particles were seen. It was suggested that the flagella consist of aggregates of protein molecules, bound and surfaced by a polyhydroxylic substance which may form resinous compounds with boric acid.

   Molybdenum.—The earlier investigations on this element have shown its essentiality for nitrogen fixation by *Azotobacter* and *Clostridium pasteurianum*. The results obtained by molybdenum treatment of certain Australian and New Zealand soils have demonstrated its beneficial effect with regard to nitrogen fixation of some leguminous crops (93). More evidence has become available that this element is of general importance in plant metabolism. Hewitt & Jones (94) obtained abnormal growth of savoy cabbage, cauliflower, mustard, and tomato when molybdenum was omitted from the nutrient medium. The deficiency symptoms in cauliflower were found to be identical with those of a disease (whiptail), found on certain acid soils in England, New Zealand, and Australia, known to be curable by adding 1 kg. of sodium molybdate per hectare (95). Walker (111) reported molybdenum deficiency in tomato grown on serpentine soils in California. Vanselow & Datta (96) obtained symptoms of molybdenum deficiency in citrus. This was the case whether the plants were supplied with nitrate or with ammonium nitrogen. Hewitt & Jones (94) observed an accumulation of nitrate in leaf tissues starved for molybdenum. Plants supplied adequately with molybdenum were found to be practically free from nitrate. Although the high nitrate content of molybdenum-deficient plants might be attributed to a reduced nitrate reduction owing to lack of carbohydrate in the chlorotic leaves rather than to a reduced activity of nitrate reducing enzymes, more evidence as to the latter suggestion was presented by the writer (97) in experiments with microorganisms (*Aspergillus niger*, denitrifying bacteria) and with tomato and barley and by Wilson & Waring (98). In agreement with the results of earlier investigations by Steinberg (99), it was shown (97) that *A. niger* requires considerably higher amounts of molybdenum when cultivated in a nutrient solution supplied with nitrate than with an ammonium salt. In experiments with four strains of denitrifying bacteria it was found that denitrification requires the presence of traces of molybdenum. To study the effect of molybdenum on nitrate reduction in higher plants, tomato plants with symptoms of molybdenum and nitrogen deficiency were supplied with nitrate. Some cultures were given traces of sodium molybdate. Harvest and analysis of the plants took place three days later when only slight color changes due to molybdenum occurred. Plants which had received no molybdenum had much higher nitrate contents and lower protein contents as compared with those treated with a trace of this element, indicating that molybdenum apparently plays an active role in nitrate reduction. Results of experiments with cauliflowers carried out by Wilson & Waring (98) and with tomato [Stout & Meagher (100)] point in the same direction. The former tested leaves from normal and molybdenum-deficient plants for nitrate and found an accumulation of it

in the mottled interveinal tissue of deficient plants. Dark green areas from molybdenum-deficient mottled leaves or from normal leaves gave either a negative or a slightly positive test for nitrate. When plants showing the interveinal chlorosis had been treated with a dilute solution of sodium molybdate, the disappearance of the nitrate from the leaf tissue preceded the development of a healthy green color. It is interesting to note that Stout & Meagher (100) in experiments with tomato observed a rapid uptake by roots of radioactive molybdenum and subsequently a rapid translocation to the leaves where it was accumulated in those regions in which loss of chlorophyll takes place in the absence of molybdenum (interveinal areas).

As to the role of molybdenum in nitrogen fixation, experiments with free-living nitrogen-fixing organisms and with leguminous plants have to be recorded. Jensen & Spencer (101) tested eight strains of *Clostridium butyricum* and found a three to sixfold increase in gain of nitrogen. Vanadium which may partly replace molybdenum in *A. chroococcum* had no influence on four strains of *C. butyricum* but gave a marked stimulation of the others. Neither *A. chroococcum* nor *Clostridium* responded to tungsten when molybdenum was absent. The stimulating effect of tungsten, described by some authors, was suggested to be due to the difficulty of separating tungsten and molybdenum. In a subsequent paper Jensen (67) dealt with the effect of molybdenum on the nitrogen fixation by *A. indicum*, a bacterium which in contrast to *A. chroococcum* may develop in acid media. In agreement with the behavior of other free-living nitrogen-fixing micro-organisms, *A. indicum* was found to require traces of molybdenum for its nitrogen fixation. In the absence of molybdenum, vanadium was entirely without effect. Apparently the nitrogen-fixing system of *A. indicum* differs slightly from that of *A. chroococcum*. When the nutrient solution had been supplied with nitrate, *A. indicum* developed normally in the absence of molybdenum. Addition of very small amounts of this element exhibited an inhibitory effect. With ammonium nitrogen no effect of molybdenum was observed. These results are opposed to those obtained with *A. chroococcum* by the writer (97). In the absence of molybdenum this bacterium grew equally poorly with gaseous nitrogen and with nitrate, but it developed normally when ammonium sulphate had been added to the solution. Assimilation of gaseous nitrogen was found to require five times more molybdenum than was the case with nitrate.

The essentiality of molybdenum in nitrogen fixation by root nodules of leguminous plants was demonstrated by the writer (97) in culture-solution experiments with pea plants. Molybdenum-deficient plants developed many nodules the color of which was pale yellow-brown as contrasted to the somewhat pinkish shade of nodules from molybdenum-supplied plants. The former did not fix nitrogen, as a result of which the plants died from nitrogen deficiency. Jensen (102) in pot experiments with *Trifolium* and *Medicago* grown in an acid sandy soil observed an increase in nitrogen fixation per unit of nodule tissue when molybdenum had been supplied to the plants. A similar effect was obtained by adding calcium carbonate without molybdenum, due

to the improved absorption of the traces of molybdenum contained in the sand, when the pH of the nutrient medium was raised. Jensen concluded that nodules of alfalfa should contain 10 to 25 p.p.m. of molybdenum and those of subterranean clover at least 7 to 8 p.p.m. in order to enable them to function normally. Responses of leguminous plants growing in natural soils to applications of molybdenum have been obtained in several earlier Australian investigations [see Anderson (103)]. In these experiments it was shown that the beneficial effect of molybdenum on pastures was due to improved nitrogen fixation of the clovers which indirectly increased the nitrogen supply to the grasses. Molybdenum deficiency due to excess of manganese in the nutrient medium as described by Millikan (104) was found by Anderson only in a single case. In a subsequent paper Anderson & Spencer (105) reported that manganese sulphate, in addition to ammonium sulphate or nitric acid, reduced the molybdenum uptake from these soils to such an extent that non-legumes responded to applied molybdenum. In pot and field experiments (unpublished) the writer obtained large responses to molybdenum of white and red clover grown on a peat soil rich in iron and on several acid sandy soils. Apparently the reduced absorption of molybdenum represents one of the causes of poor nitrogen fixation in clovers on acid soils [see also Jensen (102)].

Poisoning of cattle as a result of an excess of molybdenum in pasture plants as has been previously described by Ferguson *et al.* (106), has been found in various parts of the world. Barshad (107) studying California soils found abnormalities in cattle when the molybdenum content of the pasture plants was 20 or more p.p.m. The soils had an alkaline reaction and contained 1.5 to 10 p.p.m. of molybdenum, a high percentage of which was soluble in water. The botanical composition of the sward was found to be of much importance since different species and even different varieties of the same species are absorbing widely differing amounts of molybdenum. By eliminating plant species which absorb molybdenum to the greatest extent, it will be possible to establish wholesome pasture in affected areas. Robinson & Edgington (108) determined the molybdenum content of a number of plants grown on various soils including those of a high selenium area in South America. In the latter case high values were found. The toxicity to cattle and humans of grains and peas grown on these soils was previously attributed to the high selenium content. It seems desirable to study the possible contribution of the high molybdenum intake to these toxicity symptoms.

Aluminum.—Although it is doubtful whether aluminum has to be considered a micronutrient element for certain plants, it plays an interesting role in some biochemical reactions. When *Hydrangea* with pink flowers is transferred to an acid soil or is treated with aluminum salts, the color of the flowers will change to blue. This is due to the uptake of relatively large amounts of aluminum [Chenery (109)]. Apparently a blue-colored acid-stable aluminum lake is formed which may be regarded as a colloidal com-

plex or a loose combination of the delphinidine pigment and aluminum. According to Chenery (109) three conditions have to be fulfilled before a soil-induced color change will take place: (*a*) delphinidine pigment must be present, (*b*) the plant must be able to accumulate aluminum, and (*c*) it must have a wide range of reaction tolerance, since large amounts of soluble aluminum are only available on acid soils. In his search for plants behaving like *Hydrangea*, Chenery collected a number of species with flowers containing delphinidine derivatives. These plants were transferred to soils containing aluminum salts but they did not produce blue flowers owing to the fact that no abnormally large aluminum absorption took place. Since the acidity of the flower sap of these plants was considerably less than that of *Hydrangea* (pH 5.2 to 6.4 and 4.4 respectively), it was suggested that cell sap reaction is an important factor in aluminum uptake. In agreement with this fact it was found that a great number of aluminum-accumulating plants had an acid cell sap (pH 4.3 to 4.8). Among these plants many had blue fruits, presumably owing to the presence of an acid-stable aluminum delphinidine complex similar to that present in *Hydrangea*.

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CARBON DIOXIDE FIXATION BY
GREEN PLANTS[1]

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Introduction

Since the end of World War II when the long-lived isotope of carbon, C^{14} became available, a new tool has been applied in the study of photosynthesis. Because of the interest evoked by the tracer method, research in all areas of photosynthesis has expanded.

There have been reviews on various aspects of photosynthesis such as the primary photochemical reaction (1), quantum efficiency, products, and comparative biochemistry (2), many discussions of which were included in the monograph of The American Society of Plant Physiologists, *Photosynthesis in Plants* (3).

The discovery of the Hill reaction and its elaboration by various workers as well as the work with tracer carbon have seemed to indicate a definite separation between the carbon assimilation and oxygen evolution aspects of photosynthesis. Since a number of reviews in recent years have placed special emphasis on the oxygen evolution reaction we are going to limit ourselves here to the carbon assimilation reactions which are particularly susceptible to study with tracer carbon. We will, therefore, be concerned with such questions as: (*a*) through what sequence of compounds does carbon pass on its way from carbon dioxide to plant materials, and (*b*) what is the relationship between this process and other anabolic and catabolic processes in the plant?

Before discussing the more recent results of the tracer method it is well to describe some of the work directed toward solution of these problems by the classical methods of gross analysis for a variety of compounds in green plants which have been subjected to various conditions.

Effect of Growth Conditions on Assimilation

The effect of growth conditions on the overall composition of cell material has been the subject of much investigation and allows some conclusions to be drawn regarding the pathways of synthesis. Spoehr & Milner (4) have so adjusted growth conditions of *Chlorella* that the cells contained as much as 85.6 per cent (dry weight) fats. Protein synthesis was favored by high nitrogen nutrition and low light intensity; low nitrogen supply and high light intensity led to storage of lipids. An empirical method was developed for com-

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[1] This review covers the period approximately from June 1947 to November 1949.

paring the degree of reduction (R-value) determined by an elemental analysis and thus to calculate approximate carbohydrate, lipid and protein composition of plant tissue. Burström (5) concluded previously that nitrate reacts with intermediates of photosynthesis to form amino acids. Myers and co-workers (6, 7, 8) have studied *Chlorella* nutrition using the assimilatory quotient (carbon dioxide/oxygen) as a tool for interpreting the nature of synthetic processes occurring. Basing his empirical equations for photosynthesis upon the actual elemental composition of cells formed and their nitrogen source, Myers predicted photosynthetic quotients and obtained experimental agreement. In defining the end products of photosynthesis, Myers pointed out that their relationships are determined by the pathways of synthesis and interconversion rather than their state of reduction. Since carbohydrate is the major product of photosynthesis and the substrate for respiration in most plants, it has been convenient to consider photosynthesis as an integrated process for its production. Myers (6) and others have questioned this viewpoint and feel that the results obtained suggest that photosynthesis merges with plant metabolism prior to appearance of carbohydrates. That these relationships exist is shown in the tracer experiments described later.

Pucher, *et al.* (9) have made a careful study on the compounds present in leaves of *Bryophyllum calycinum* as a function of conditions under which the leaves were collected. Increases of starch and hexose content during the day and of total organic acids (largely malic) during the night were observed. Oxidation in the dark via the Krebs tricarboxylic acid cycle seemed apparent to these workers from their results and is comparable to the dark respiration of photosynthetic intermediates discussed below.

The earlier work of Smith (10) had demonstrated that sunflower leaves store sucrose and starch. Brown (11) studied the effect of prolonged photoreduction upon the carbohydrate content of *Scenedesmus*. He found decreased soluble saccharides and no change in insoluble polysaccharide content after a thirty-hour period of photoreduction. He concluded that the storage products of photoreduction are not carbohydrate as had been inferred from the assimilation quotient. It appears reasonable that algae should form protein and fats in large amounts for future growth rather than the carbohydrates formed in higher plants as energy sources for translocation to non-photosynthetic tissues. Brown's results might have been more meaningful had he compared algae which had photosynthesized in carbon dioxide with those which had photoreduced carbon dioxide with identical times, light intensities, media, etc.

ABSORPTION OF CARBON DIOXIDE BY ISOLATED CHLOROPLASTS

Reduction of carbon dioxide has not been observed during oxygen evolution by isolated chloroplasts. Boyle (12) reported that minute amounts of carbon dioxide are necessary for oxygen evolution but Brown & Franck (13) and Aronoff (unpublished) observed no significant $C^{14}O_2$ fixation under a variety of conditions. Bolchenko (14, 15) reported that a hydrogenase sys-

tem in chloroplast preparations obtained from a number of plants converts carbon dioxide in the presence of hydrogen to a substance capable of reducing mercuric ion. The nature of the reducing substance was not determined although its formation paralleled carbon dioxide uptake.

METABOLISM OF ADDED INTERMEDIATES

Another classical approach in the elucidation of chemical reactions involved in photosynthesis involves feeding suspected metabolic substrates to the intact plant. Experiments such as those of Myers (16) on the oxidative assimilation of acetate and glucose in *Chorella*, of Algeus (17) on glycine assimilation in *Scenedesmus*, and of Kolesnikov (18) on glycolic acid oxidation by barley *brei* are typical of this approach. Unfortunately cell membranes are not readily permeable to many important intermediates such as phosphate esters and are permeable to carboxylic acids only at low pH. Experiments describing the metabolic fate of isotopically labeled substrates are more conclusive since small amounts of assimilated compounds may be readily studied. Krotkov and Barker (19) studied the assimilation of radioactive acetate and found it taken up into water-soluble subtances, prior to respiration as carbon dioxide. Similar dark experiments with tobacco leaves by Tuttle (20) have shown that labeled acetates become incorporated in malic and citric acids in a manner consistent with the existence of a tricarboxylic acid cycle. The carboxyl groups of acetate or glycine were readily respired while the methyl carbon of acetate was respired slowly and that of glycine not at all. From this evidence, acetate *per se* was not believed to be an intermediate in normal respiration.

C^{14}-labeled sugars, carboxylic acids and amino acids are increasingly available. A number of feeding experiments with them during photosynthesis have been performed in the writers' laboratory and the resulting soluble metabolic intermediates have been separated by paper chromatography. The results obtained provide additional support for the proposed CO_2 assimilation processes. Although present methods allow a simple and rapid determination of metabolic products there remains, however, the problem of getting the metabolite into the cell in the biologically active form and proving that it reacts exactly as the natural substrate. Of all substrates, labeled carbon dioxide is the only one free of such equivocation and its use has produced much of the present knowledge of the path of carbon in photosynthesis.

INTERMEDIATES OF CARBON DIOXIDE REDUCTION

The earliest work with the tracer method was that of Ruben and co-workers which extended over the period of 1938 to 43. Most of this work has been reviewed previously; it will be discussed here only insofar as the more recent results bear on its interpretation.

Ruben, Kamen & Hassid (21), examined the products of short photosynthesis (1 to 5 min.) and found evidence for the absence of many compounds identified in our experiments. Of the compounds Ruben added as carrier, no

activity was found in the following: pyruvic, glyceric, succinic, malic, citric, fumaric, aspartic and glutamic acids; alanine, serine and glycine; glucose, fructose and sucrose. When diffusion and sedimentation rates of the radioactive products were measured the molecular weight appeared to be~1,000. In the light of present results it is not exactly clear why substances such as malic acid and alanine were not found active. It is possible that adsorption of such compounds on large molecules affected molecular weight determinations. It is now recognized that co-precipitation methods such as those used by Ruben *et al.* are unreliable, especially when used to separate more than one substance at a time from the same solution. Unfortunately these workers had not included phosphoglycerate or hexose phosphates as carriers when examining known compounds for radioactivity. Although none of the early intermediates had been identified, Ruben arrived at the conclusion that there was a primary carboxylation reaction to form RCOOH which was followed by a photochemical reduction which regenerated the carbon dioxide acceptor RH and thus resulted in carbon dioxide assimilation. He assumed that carbon dioxide was fixed anaerobically in the dark by *Chlorella* in a compound, RCOOH, which was subsequently reduced during photosynthesis. The first available C^{14} was used in an effort by Ruben and one of us (Benson) to isolate this compound. This work was continued at Berkeley after World War II and led to the isolation of succinic acid (22) as the major product. Succinate had been identified as a $C^{11}O_2$ fixation product by the protozoan, *Tetrahymena geleii* by van Niel *et al.* (23) and in the fermentation of glycerol in $C^{11}O_2$ by *Propionibacterium pentosaceum* by Carson & Ruben (24).

Determination of the path of carbon in photosynthesis.—In view of the fact that both Ruben and others had demonstrated the fixation of carbon dioxide in the dark by a variety of organisms it appeared desirable to perform experiments in such a way that the method of carbon dioxide fixation was unequivocally that of photosynthesis. This quite clearly entailed the feeding of radioactive carbon dioxide to the organisms in an active state of photosynthesis in the light and studying the sequence of intermediates through which the carbon passes. The results of such experiments have so far been published by four groups of workers and will be discussed from the point of view engendered by our own work.

Algae[2] or leaves were first allowed to photosynthesize in $C^{12}O_2$. Radioactive sodium bicarbonate or $C^{14}O_2$ was then rapidly added in an amount small enough not to disturb the steady state concentrations of metabolites. The partial pressure of carbon dioxide should have changed little during the experiment. After the chosen period the plants were killed as rapidly as possible, usually in boiling ethanol. In short photosynthesis experiments (less than 1 min.) almost all of the radioactive products were soluble in 80 per cent ethanol (25). For similar experiments Brown, Fager & Gaffron (26) have reported complete curves for the relative amounts of $C^{14}O_2$ converted to fats and benzene-soluble pigments (fraction A), alcohol soluble compounds

[2] We are indebted to Professor Gaffron for the strain of *Scenedesmus* (D_3) used in this work.

(fraction B) and insoluble material such as denatured proteins, cellulose and starches (fraction C). These workers did not state the probable components of their extracts and residues. In their shortest exposures to $C^{14}O_2$, all of the fixed radioactivity was found in fraction B.

Separation of intermediates.—Ion exchange resin separations (22, 25, 27, 28) allowed the separation and identification of alanine, phosphoglyceric acid, and equal amounts of glucose and fructose as some of the products of short photosynthesis experiments. Phosphoglycerate is unique among the major products of short photosynthesis in that its two acidic groups increase its affinity for the anion exchange resins. It is less readily eluted than any other compound stable toward mild acid hydrolysis. This property was used to obtain almost pure samples in tracer amounts for comparison with authentic phosphoglycerate (28). Personal communications received from Drs. Gaffron & Fager indicate that similar techniques have been successfully applied in the Chicago Laboratory for the isolation and identification of macroamounts of phosphoglyceric acid as a major constituent of fraction B in short photosynthesis experiments.

Silica gel partition chromatography has been applied by Benson et al. (25) and by Burris et al. (29) for the separation of carboxylic acids formed by plants during photosynthesis in $C^{14}O_2$. Both reported major fractions of activity incorporated in malic acid. The results reported by Fager (30) indicate that malic acid was present but was not identified.

Burris, Wilson & Stutz (29) reported specific and total activities in carboxylic acids formed from $C^{14}O_2$ during both light and dark assimilations by *Bryophyllum*, tobacco, tomato and barley leaves. The interconversion of these products in a subsequent dark period was reported. Malic acid was not observed to decrease while citric and isocitric acids increased markedly in the dark. There appeared to be no conversion of malic acid to citric acid. This would seem to indicate that activity in these respiration intermediates is derived from sources other than the carboxylic acids formed in the light. In 30-min. photosynthesis by barley, 84 per cent of the carboxylic acid activity appeared in malic acid with less than 10 per cent in any other carboxylic acid. In a 15-min. dark fixation the products included 43 per cent succinic acid and 47 per cent malic acid as well as over twice as much citric acid and isocitric acids as in the light experiment. These results seem to be added evidence that newly reduced carbon does not rapidly accumulate in respiration intermediates in the light and that malic acid is probably closely related to the intermediates of photosynthesis as well as being involved in respiratory reactions.

Paper partition chromatography (31) of plant extracts for determination of amino acid constitutents (32) sugars (33) and protein hydrolysates have been reported. Fink & Fink (34) applied autoradiography in detecting radioactive products on paper chromatograms of compounds synthesized by *Chlorella*. Since their algae photosynthesized for 4 hours in $C^{14}O_2$ the products are not directly pertinent to a discussion of the mechanism of carbon dioxide reduction. Stepka, Benson & Calvin (35) reported the results of similar tech-

niques on extracts which had photosynthesized for 30 seconds or had fixed $C^{14}O_2$ in the dark immediately after preillumination. In both cases alanine and aspartic acid were found to be the major radioactive amino acids. No labeled glutamic acid was found.

In determining the sequence of appearance of intermediates it will be helpful to plot activity in each intermediate as a function of time. As each reservoir becomes saturated with radioactive carbon the slope of the plotted curve will approach zero. Comparison of a set of such curves should be evidence for the sequence of synthesis. In order to simplify the study of synthetic sequence low temperature and low light intensity should slow down the processes involved and simplify the experimental problem.

Paper chromatography allows rapid and complete separation of many other compounds involved in photosynthesis (35, 36, 37). The activity in each substance may be determined by counting the area defined by the radiogram (radioautograph of paper chromatogram) with a large window Geiger-Müller tube. Data so obtained were tabulated by the writers (38).

Phosphoglyceric acid was the major product in our experiments of shortest duration (5 sec.). A small amount (25 per cent) of phosphopyruvate appeared in all cases. Which of these compounds results from addition of $C^{14}O_2$ to a two-carbon acceptor is a subject for further investigation. Triose phosphate has been observed in 15 sec. experiments while in longer exposures its percentage of total activity becomes small. The total amount of hexose diphosphate observed is also considerably smaller than that of phosphoglycerate even after several minutes. Fructose- and glucose-6-phosphates have not yet been separated satisfactorily. It is known that these compounds lie in an area on the chromatogram containing hexose monophosphates.

The sucrose synthesized in 30 sec. by *Chlorella* was hydrolyzed and the specific activity of fructose was twice as high as that of glucose (36). This suggests that synthesis of fructose structures precedes synthesis of glucose phosphates. In *Chlorella* after 90 sec. the two hexoses in sucrose have nearly equal activity. The nature of the reactions immediately prior to formation of free sucrose is not yet known. No free hexoses appeared in 5-min. experiments with algae, sugar beet, barley, or geranium. It is necessary to assume then, that sucrose is formed either by simultaneous condensation and dephosphorylation of two hexose phosphate molecules or by dephosphorylation of a sucrose phosphate. No sucrose phosphate has yet been detected.

High molecular weight products.—A number of dextrins have been observed, particularly in experiments with *Bryophyllum* leaves and algae (37). Algae which have photosynthesized for 2 min. and then were exposed anaerobically to light for 3 min. in the absence of carbon dioxide before killing have been observed to form a considerable quantity of such dextrins. Although the molecular weight of the dextrins has not yet been investigated there is evidence that they are simple two, three, four and five glucose structures.

The major portion of insoluble products formed in the first few minutes by algae in the authors' laboratory (unpublished) was protein. Acid hydrolysis produced radioactive amino acids in approximately the same relative

amounts as those in the cell extract. Protein obtained from longer experiments (5 to 10 min.) contained more activity than that found in several amino acids present in the cell extracts. Glutamic acid, by far the largest free amino acid reservoir, was not converted into protein in amounts commensurate with its concentration. The early insoluble products formed by barley were largely carbohydrates as shown by their hydrolysis to glucose and levulinic acid.

Chemical identification.—The methods of chemical identification of these intermediates have been reported in a series of papers from this laboratory (25, 28, 37). The radioactive products were separated at first by virtue of their adsorption properties on exchange resins and identified by chemical properties, distribution coefficients and co-crystallization.

Carboyxlic acids such as malic, succinic, fumaric, glyceric and glycolic acids were identified by co-chromatography with synthetic radioactive acids (37). The major compounds involved in sucrose synthesis were shown to contain phosphorus by comparison with the compounds photosynthesized with radiophosphate. They included some of the known glycolysis intermediates which were prepared with radiophosphate. Paper chromatographic separation of phosphate esters has been described by Benson *et al.* (37), Cohen (39) and by Hanes & Isherwood (40). In addition to co-chromatography of 3-phosphoglycerate with that formed by plants its direct isolation from extracts of *Scenedesmus* has been described (37). Over 65 per cent of the radioactivity fixed by *Scenedesmus* in 5 sec. was isolated in the barium salt of phosphoglyceric acid. Periodate oxidation of glyceric acid formed by enzymatic or acid hydrolysis gave the required amount of activity in formaldehyde, formic acid and carbon dioxide or in glyoxylic acid and formaldehyde.

Fager (30) has reported results of tests on the radioactive components of fraction B as defined by Brown, Fager & Gaffron (26). Our own data indicate that this water-soluble fraction contains a multitude of compounds involved in photosynthesis and that very few occur to the extent of more than ten per cent in the mixture. Fager proceeded to concentrate the major photosynthetic intermediate with several assumptions in mind. The first was that the major portion of tracer was fixed in only one or two compounds which are not related to normal respiration intermediates. The second assumption was that fraction B was resistant to utilization in cell metabolism by respiration or other metabolic reactions.

The argument for the simple composition of fraction B (26) (40-sec. photosynthesis) rested on the apparent nonsigmoid character of the C^{14}-in-fraction B assimilation curve. Brown, Fager & Gaffron (26) observed no appreciable lag in formation of fats and insoluble material. The adsorption of low-molecular intermediates on proteins may have caused the appearance of activity in the insoluble material in the shortest experiments. Our experience is that phosphoglycerate is such a strongly adsorbed compound. These workers did not expect that even in experiments as short as 40 sec. one might obtain a large number of radioactive intermediates. The sigmoid character of the curve for fraction B curve might appear only when investi-

gated at shorter times and when the actual nature of the insoluble activity in fraction C is known.

The uniqueness of fraction B in metabolic reactions was assumed from the fact that in the dark radioactivity in fraction B was not converted to fats or insoluble material. These conversions are not the only possible results of changes in radioactivity of fraction B. We have observed that phosphory-lated intermediates of hexose synthesis are rapidly converted to sucrose and to the Krebs tricarboxylic acid cycle intermediates of respiration (38). With algae, sucrose is respired while in barley leaves the degradation of sucrose is very slow compared to that of the intermediates of its synthesis. It is thus apparent that the simple nature (i.e. one or two compounds) of the radio-activity in fraction B and its resistance to transformation (stability) save under the influence of light, need not be inferred from its nonconversion to insoluble material in the dark.

In the course of determining the chemical properties of fraction B, Fager (30) carefully examined all the reasonably expected metabolic intermediates for radioactivity. Of these none was found radioactive. The major difficulty in the isolation work reported seems to us to have been the dilution of a small amount of radioactivity with tremendous amounts of plant material. Experi-ments such as the benzoylation of amino acids are typical. An active extract containing 1,960 counts per minute (cpm.) of C^{14} was benzoylated and 663 mg. of products was examined for radioactivity. The average specific activity, 3 cpm. per mg., is very low compared to the maximum obtainable, 10^6 to 10^7 cpm. per mg., and impedes identification procedures. The amino acids present would have given such small amounts of benzoyl derivatives that they would have been water-soluble and would not have appeared in the in-active precipitate reported by Fager. Only solvent extraction of benzoyl-amino acids which yielded an active extract seems of significance and in-dicates 15 per cent of amino acids which corresponds to our experiments. Silica gel partition chromatography was used by Fager and indicates pos-sible 23 per cent of dicarboxylic acids such as malic. The evidence presented for the absence of known metabolic intermediates cannot be accepted as pre-cluding the existence of moderate amounts of many of them.

Degradation of intermediates and products.—The distribution of labeled carbon within the molecule is valuable evidence for defining the path by which the compound was formed. Such evidence has been applied in the study of a great number of biochemical processes. Any proposed reaction mechanism must be verified both by the relative sequence of appearance of the intermediates and by the distribution of newly assimilated carbon within the intermediate. The degradation data which have accumulated in the past few years are the basis of the proposed reaction sequence for photosynthesis given below.

The hexose degradation method developed by Wood, Lifson & Lorber (41) has been widely applied (42, 43, 44). Lactic acid fermentation followed by chemical degradation gives the relative isotope concentrations in the 3-4, 2-5, and 1-6 pairs of carbons. No evidence has been reported for asym-metric distribution of newly incorporated carbon. Wood & Burr (45) applied

this degradation procedure to hexose synthesis by bean leaves from $C^{13}O_2$ and found some variations in C^{13} content. Aronoff, Barker & Calvin (42) reported unequal distribution of C^{14} (61 per cent, 24 per cent, 15 per cent in carbon atoms 3-4, 2-5, 1-6 respectively) in barley hexoses formed during 40-min. photosynthesis. No satisfactory explanation for unequal C^{14} distribution in an experiment as long as 40 min. has yet been advanced. Similar results were obtained for hexoses synthesized in 30 sec. (25).

Gibbs (43) reported results of degradation of sucrose isolated after long photosyntheses by canna and barley in which the hexoses were uniformly labeled. However, with monosaccharides (sucrose was not subject to the fermentation) from 1-hr. photosynthesis by barley in $C^{14}O_2$ Gibbs found a distribution of 15 per cent, 28 per cent, 56 per cent in the 3-4, 2-5, 1-6 positions of the hexose respectively. Such a distribution is most probably caused by a subsequent short period of photosynthesis in $C^{12}O_2$ during the time of opening the photosynthesis chamber and killing the plants. Such results have been obtained previously (25) as a result of photosynthesis of low specific activity carbon dioxide at the end of a long experiment. Gibbs (46) found a distribution of 51 per cent, 30 per cent, 19 per cent in positions 3-4, 2-5, 1-6 in hexoses of sunflower leaves which is in agreement with that of Aronoff[3] for 90-sec. photosynthesis by soybean leaf (49 per cent, 23 per cent, 19 per cent in positions 3-4, 2-5, 1-6) and with the results obtained in the writers' laboratory.

It has become apparent that algae and the higher plants differ considerably in the rate at which hexoses become uniformly labeled. The sequence of labeling, however, is the same in all plants investigated. The hexoses and phosphoglycerate synthesized by barley leaves in periods longer than 60 sec. are uniformly labeled. For *Scenedesmus* uniform labeling requires 3 to 5 min.

In 5 sec. photosynthesis by *Chorella* or *Scenedesmus* 5 per cent of the C^{14} in phosphoglycerate was found by Bassham[4] to be in the α and β carbon atoms. Barley had 15 per cent in the α and β carbons of phosphoglycerate after only 2 sec. of photosynthesis. The distribution of C^{14} in alanine corresponds to that in phosphoglycerate and in the hexoses. When malic, succinic or aspartic acids were degraded the distribution corresponded to that derived by addition of $C^{14}O_2$ to a three-carbon compound, as in the Wood-Werkman reaction. Both carboxyl groups were labeled with high specific activities and the central carbon atoms are labeled to the extent of that found in the α and β carbons of three-carbon compounds or in the 2-5 and 1-6 positions of the hexose.

A mechanism has been proposed (25) to account for the observed compounds and their isotopic distribution. It is essentially a dicarboxylic acid cycle in which a two-carbon acceptor molecule is converted to oxalacetate by two successive carboxylations. Upon splitting the four-carbon acid, two new acceptor molecules are formed. The intermediates of photosynthesis are diverted from this cycle for synthesis of fat, amino acids and carbohydrate.

[3] S. Aronoff, personal communication.
[4] J. A. Bassham, personal communication.

$$C_2 \text{ acceptor } [CH\text{=}CH\text{-}O\text{-} \textcircled{P} \text{ ?}] \xrightarrow{C^{14}O_2} \underset{\text{2-phosphoglyceric acid}}{\overset{\overset{\displaystyle OH}{|} \overset{\displaystyle O\text{-}\textcircled{P}}{|}}{CH_2\text{-}CH\text{-}C^{14}O_2H}} \longrightarrow \text{3-phosphoglycerate}$$

Benson and Calvin carbon dioxide fixation cycle diagram
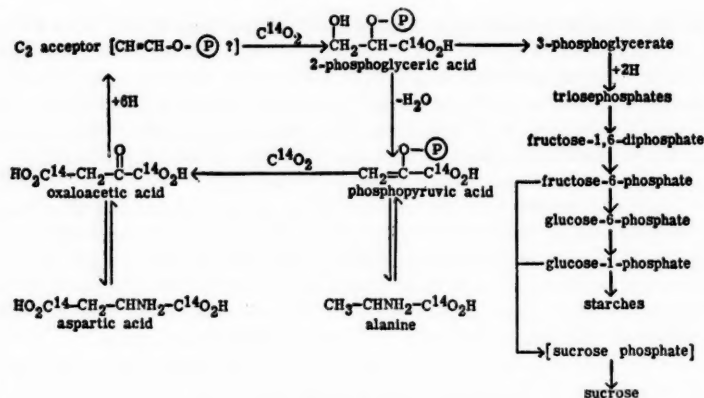
CARBON DIOXIDE FIXATION CYCLE

The phosphoglycerate formed initially is carboxyl-labeled. When a carboxyl-labeled structure has passed through the cycle once, the newly-formed acceptor is labeled in the reactive end. Subsequent α-carboxylation gives phosphoglycerate labeled largely in the carboxyl but also in the α position to an extent determined by the size of the reservoirs of intermediates in the cycle and by the duration of the synthesis. A second passage around the cycle, followed by α-carboxylation yields phosphoglycerate labeled with increased amounts of isotope in the β, α and carboxyl carbons. When such a compound is converted to sucrose similar isotope distribution occurs in the 1-6, 2-5, and 3-4 carbon atoms, respectively, of the hexoses. The size of the reservoirs of intermediates in the cycle, as well as those in rapid equilibrium with intermediates greatly affect the rate of attainment of uniform isotope distribution.

The proposed cycle is written without specifying the exact nature of the conversion of oxaloacetate to two-carbon acceptor. Originally (22) it was assumed that oxaloacetate might be converted by well-known reactions into acetate through malate, fumarate and succinate and a reductive splitting of succinate. The apparent absence of succinate and fumarate in the synthetic paths used by higher plants led to serious doubts regarding their participation.

The role of glycolic acid in plant metabolism.—Kolesnikov (47, 48) has shown that glycolic acid may act as a substrate for oxidation by suspensions prepared from barley leaves. He also observed a catalytic oxidative effect of glycolic acid similar to that reported earlier by Anderson (49) for the colorless alga *Prototheca zopfii*. Kolesnikov (18, 48) presented evidence that organic peroxides responsible for such oxidation were formed from glycolic and glyoxylic acids and that both acids acted catalytically in the oxidation of chlorophyll. The observation that this oxidative system was obtainable only from chlorophyll-bearing tissues led him to suggest that it is an element of the photosynthetic system.

Tolbert, Clagett & Burris (50, 51) have purified a widely distributed enzyme obtained only from chlorophyll-containing tissues which catalyzes oxidation of L-α-hydroxy acids. Glycolic acid is oxidized via glyoxylic acid to formate and carbon dioxide. Lactic acid is oxidized to pyruvic acid. With crude enzyme preparations or tobacco leaf sap the oxidation proceeds through glyoxylic acid, but to products other than formate and carbon dioxide.

Glycolic acid has been observed as a product of short photosynthesis by all plants studied in the writers' laboratory (38) and by those studied by Burris, Wilson & Stutz (29) excepting *Bryophyllum*. It appears as a major product ($\sim$ 25 per cent of total radioactivity) during strong illumination, low carbon dioxide pressure and aerobic conditions. Conditions such as these for optimal glycolic synthesis correspond to those for photooxidation described by Franck & French (52) who suggested formation of a carboxylic acid during the process. Glycolic acid is also observed in barley extracts after photosynthesis on $C^{14}O_2$ followed by a period of illumination with nitrogen flushing. Since glycolic acid is found at low oxygen pressures, even though it be in smaller amounts, one is led to suspect its participtaion in the synthesis of the two-carbon acceptor of photosynthesis. The appearance of glycine simultaneously with that of glycolic acid, lends support to the participation of glyoxylic acid as well. It remains to be demonstrated conclusively whether glycolic acid arises as a reservoir for two-carbon acceptor molecules or whether its presence is wholly due to photooxidation of such acceptors.

The appreciable amounts of glycine and glycolic acid formed even in short experiments suggested the possibility that the splitting occurred in a four-carbon acid more oxidized than succinic acid. Dihydroxymaleic acid, the oxidation of which was investigated by Kuzin & Doman (53) may play a role in the process but neither this acid nor its precursor, tartaric acid, has been isolated or identified. This possible mechanism (54) is given below.

$$
\underset{\text{oxaloacetic acid}}{HO_2C-CH_2-\overset{\displaystyle O}{\overset{\|}{C}}-CO_2H} \rightarrow \underset{\text{enol-oxaloacetic acid}}{HO_2C-CH=\overset{\displaystyle OH}{\underset{|}{C}}-CO_2H} \overset{H_2O}{\longrightarrow} \underset{\text{tartaric acid}}{HO_2C-\overset{\displaystyle OH}{\overset{|}{C}H}-\overset{\displaystyle OH}{\overset{|}{C}H}-CO_2H} \overset{-2H}{\longrightarrow}
$$

$$
\underset{\text{dihydroxymaleic acid}}{HO_2C-\overset{\displaystyle OH}{\overset{|}{C}}=\overset{\displaystyle OH}{\overset{|}{C}}-CO_2H} \rightarrow \underset{\text{ketomalic acid}}{HO_2C-\overset{\displaystyle OH}{\overset{|}{C}H}-\overset{\displaystyle O}{\overset{\|}{C}}-CO_2H} \rightarrow \underset{\text{glyoxylic acid}}{HO_2C-CHO} \overset{+2H}{\longrightarrow}
$$

$$
\underset{\text{glycolic acid}}{HO_2C-CH_2OH} \rightarrow C_2 \text{ acceptor}
$$
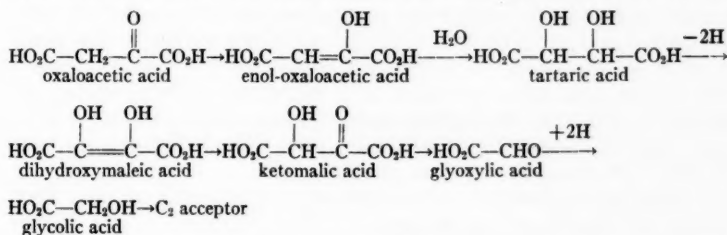
A path such as this seems reasonable on the grounds that it involves only simple reversible hydrations, hydrogenations and formation of carbon-carbon bonds by benzoin-type condensations, all of which are well-known in biosynthesis.

The works of Anderson, Kolesnikov, and Clagett, Tolbert & Burris are in agreement with the writers' results and those of Burris, Wilson & Stutz

showing that radioactive as well as inactive glycolic acid disappears rapidly in the dark. This rapid disappearance before the plant is killed accounts for the previous absence of conclusive identification of glycolic acid in plants.

Dark Fixation of Carbon Dioxide

The dark fixation of $C^{11}O_2$ in *Chlorella* and barley was observed by Ruben, Hassid & Kamen (21). These experiments have been repeated by Brown, Fager & Gaffron (26) and by Benson *et al.* (37) with *Scenedesmus*. The results of the three groups are nearly identical except that Ruben's curve (21) showed that dark fixation reaches a saturation point. Actually a slow dark uptake proceeds for many hours. Brown *et al.* (26) confirmed the earlier finding that such dark fixation is a reversible process but discounted Ruben's supposition and that of Allen, Gest & Kamen (55) that such dark fixation is related to photosynthetic fixation except for some possible very unstable and rapidly dissociable product.

These experiments are readily interpreted in view of the nature of products formed (38). *Chlorella*, *Scenedesmus* and barley incorporate $C^{14}O_2$ in the dark into carboxyl groups of glutamic, citric (iso), succinic, fumaric, aspartic and malic acids as well as in alanine. These compounds, related to, or members of, the Krebs tricarboxylic acid cycle, are apparently universally involved in plant as well as in animal respiration as is shown by the works of Bonner, Vennesland, Weinhouse and others. The rate of dark fixation is constant except for an initially rapid uptake, presumably caused by previous depletion by pumping or by flushing with a carbon dioxide-free gas.

Carbon dioxide fixation by succulents.—Bonner & Bonner (56) determined the rate of acid accumulation in leaves of several succulents as a function of temperature and carbon dioxide pressure. They observed a rapid linear rise in acid accumulation to 0.1 per cent carbon dioxide followed by a much slower linear rise up to concentrations of 10 per cent carbon dioxide, not unlike the effect on photosynthetic rate of other plants or the effect on dark fixation rate of preilluminated *Scenedesmus* (57). The nature of the acids formed during the relatively large scale dark fixation of $C^{14}O_2$ by *Bryophyllum crenatum* was determined by Bonner & Thurlow (58). The amount of radiocarbon fixed in the leaves was nearly twice that simultaneously respired and twice the net fixation. The fraction including isocitric, citric, and malic acids contained half the fixed activity. These are the same compounds as those observed in dark fixation by algae and barley (38).

Enhancement of dark fixation by preillumination.—Seeking enhanced dark fixation for isolations of the compounds formed, the writers (28, 37) illuminated plants anaerobically in the absence of carbon dioxide. The result was a ten- to one-hundred-fold increase in initial dark fixation rate, followed by a slow normal fixation rate. The dark fixation ability increased to a maximum with preillumination. *Scenedesmus* required 1 or 2 min. of preillumination to reach maximum dark reduction ability; *Chlorella* was several times slower. This phenomenon was interpreted (28) as a formation of "reducing power," such as a reduced coenzyme, or any other reduced compounds

by preillumination which could later reduce a limited amount of carbon dioxide in the dark. This reducing power was observed to decay in the dark ($t_{1/2}=\sim2$ min.) due presumably to reduction of respiratory or fermentation carbon dioxide or other reducible substrates, and could be repeatedly restored by illumination. It should be pointed out that this phenomenon is diminished at very high light intensities.

This interpretation has been criticized by Brown, Fager & Gaffron (26) and by Franck[6] who interpret the result as a restoration of equilibrium shifted by photosynthetic depletion of carbon doxide during preillumination. The analyses of Calvin & Benson (28) show considerable increase of activity in alanine after dark fixation, as a function of preillumination. However, radioactive glutamic and citric acids are not formed under these conditions. Thus the depletion and restoration of the alanine reservoir by preillumination and dark fixation does not indicate shifts in respiratory intermediates, but rather in photosynthetic ones. Most convincing evidence for the direct relationship between preilluminated dark fixation and photosynthetic fixation lies in the identity of compounds formed in the two cases (38). Considerable quantities of sucrose and intermediates in its synthesis have been formed in the dark by preilluminated *Chlorella* and barley leaves. Most of the carbon dioxide absorption occurs in the first half minute of such fixation. More time, from one to two minutes, is required for sucrose synthesis from its phosphorylated precursors.

The possibility that enhanced dark fixation could have been due to a mass action reversal of respiratory or fermentative decarboxylations was considered by the writers (57). The dependence of dark fixation on carbon dioxide pressure was determined for normal and preilluminated *Scenedesmus*. The curve for preilluminated cells, which resembled that for the dependence of photosynthetic rate on carbon dioxide pressure, had an initial slope 100 times greater than that for dark fixation by cells without preillumination. Because of compensation by fermentation or respiration, the carbon dioxide partial pressure within the cell cannot reasonably be expected to decrease one hundred-fold (i.e. to 0.001 mm.) during preillumination; it was thus concluded that the major action of light was to produce reducing agent(s) and carbon dioxide acceptor(s).

The distribution of C^{14} within the intermediate molecules also shows the nature of this dark fixation. Only carboxyl-labeled compounds would be expected from reversal of known fermentation or respiration reactions. Degradation data accumulated in this laboratory (25) demonstrate the presence of 4 to 10 per cent of C^{14} in carbons other than carboxyl groups in alanine, phosphoglycerate and succinate, as well as in the 2-5 and 1-6 carbons of the hexoses. While it is possible that such a result is due to some as yet unknown sequence of side reactions, the writers feel that such labeling may best be explained as an actual operation of the carbon dioxide-acceptor regenerating cycle described earlier.

[6] Franck, J. Remarks at A.A.A.S. Symposium on Photosynthesis December, 1947.

Differential inhibition of respiration and dark carbon dioxide-fixation.—
The earlier cyanide inhibition experiments of Ruben, Kamen & Hassid (21)
were extended by Allen, Gest & Kamen (55) who used both *Chlorella* and
Scenedesmus. This work has been criticized extensively by van Niel (2) who
concluded that the evidence presented does not preclude photosynthetic
carbon dioxide fixation via some reversible step in the Krebs cycle. The mag-
nitude of the dark $C^{14}O_2$ fixation in the presence of cyanide was shown to
parallel photosynthetic activity and to be independent of endogeneous
respiration in both algae. Previous starvation reduced dark fixation by
Scenedesmus but not by *Chlorella*. These results did not convince Brown,
Fager & Gaffron (26) that the dark fixations observed were in any way re-
lated to the dark pick-up which must precede photosynthesis. They sug-
gested that dark fixation by cyanide-inhibited cells is comparable to anaero-
bic dark fixation and to at least the first stages of aerobic dark fixation.

While no conclusions may definitely be drawn regarding the influence of
cyanide on the processes of dark fixation, identification of the various
products of aerobic and anaerobic dark fixation shows that they are largely
intermediates of the tricarboxylic acid cycle. Unpublished experiments with
Scenedesmus performed in the writers' laboratory show that it differs from
Chlorella (38), in that *Chlorella* forms no appreciable amount (<2 per cent)
of phosphorylated glycolysis intermediates, while *Scenedesmus* forms up to
24 per cent of radioactive phosphate esters during a 40-min. dark fixation.
Under aerobic conditions the amount of phosphates and succinic acid is
small while the fraction of glutamic acid is large (20 per cent). Succinic acid
is the major anaerobic dark fixation product, while under aerobic conditions
succinic, malic, and glutamic acids are major products. The major products
common to both dark and photosynthetic fixations are alanine, aspartic
acid, and malic acid. It should now be possible to compare experimentally
the products of dark fixation by normal and cyanide-inhibited *Scenedesmus*.
It may well be that uptake of C^{14} into tricarboxylic acid cycle intermediates
may continue while all incorporation into phosphates involved in carbo-
hydrate synthesis is inhibited. Such a result would be in fair agreement with
the results of Allen, Gest & Kamen and would be added evidence that all
reactions from carbon dioxide to carbohydrate are reversible and require no
simultaneous photochemical step.

Relation of respiration to photosynthesis.—All experiments designed to
measure the rate of light respiration have involved various assumptions.
The selective poisoning experiments of Gaffron (59) with *Scenedesmus* have
their counterpart in those of Warburg (60) with *Chlorella*. It seems unlikely
that true selective poisoning of either photosynthesis or respiration can be
obtained since the intermediates and types of reactions are so closely related.

Experiments at low carbon dioxide pressures where gas exchange at con-
stant light intensity may be extrapolated to zero carbon dioxide pressure to
determine the light respiratory rate have been performed by Hoover, John-
ston & Brackett (61), Gabrielsen (62), and Warburg *et al.* (63). Such experi-
ments have not yet yielded reliable data for extrapolation. Gabrielsen (64)

measured the carbon dioxide content of gas which rapidly flowed past illuminated sun leaves (thick) and shade leaves (thin) of elderberry. Although the shade leaves were found to evolve about as much carbon dioxide in the light as in the dark the respiration of sun leaves was reduced about one-half in the light. Gabrielsen interpreted the decrease as due to reassimilation of respired carbon dioxide since the amount rephotosynthesized diminished with increasing gas velocity and decreasing respiratory rate. If this were true, it would mean that respiratory intermediates must be converted to carbon dioxide before reassimilation by photosynthesis. For similar reasons, Burk *et al.* (65) found it necessary to agitate their algae violently in order to prevent reassimilation of respiratory carbon dioxide. Kok (66) measured oxygen exchange near the compensation point as a function of light intensity and extrapolated the light dependence of photosynthesis to zero intensity. As Weigl (67) has pointed out, Kok's data from which he concluded that light respiration was one-half that in the dark could be interpreted to show an increase of respiration at high light intensities. It is difficult to extrapolate from measurements at extremely low carbon dioxide pressures or low light intensities to the relationships prevailing under more normal conditions.

Weigl (67) fed isotopic carbon dioxide to barley leaves and followed the dilution of the gas phase radioactivity by inactive carbon dioxide from respiration. He found that light decreased the respiratory evolution of carbon dioxide, but was unable to show whether this was due to a real decrease in respiration or merely another case of reassimilation of the carbon dioxide before it reached the gas phase. It would seem that gas phase measurements alone cannot give a true value for either carbon dioxide assimilation or respiration in the light.

The question of the availability of photosynthetic intermediates for respiration has also been partially answered by his experiments (67). Intermediates formed from $C^{14}O_2$ were not respired while the light was on. As soon as the light was turned off the specific activity of the gas rose rapidly, indicating that the newly formed intermediates were being respired. Reillumination reduced the specific activity as well as the total carbon dioxide to a low value. The rise in specific activity of the gas phase could again be observed when illumination ceased. These results are in accord with results observed on radiograms of plant extracts (38). When the light is on, whether carbon dioxide is present for photosynthesis or not, the intermediates of previous photosynthesis with $C^{14}O_2$ are only slowly respired through tricarboxylic acid cycle intermediates. However, when illumination is decreased or ceases, the radioactive intermediates of sucrose synthesis are rapidly respired through the tricarboxylic acid cycle.

In his interpretation of the contradictory results in quantum yield measurements Franck (68) proposed that two entirely different photosynthetic processes may occur. He concluded that chloroplast membranes may be permeable to respiratory intermediates at low pH and impermeable at high pH. The experiments performed in the writers' laboratory with a variety of leaves and algae at pH 4 and with algae up to pH 8.7 have shown no detect-

able change in the nature of the intermediates. It is unlikely that moderate changes in the external pH affect the pH near the chloroplasts.

CONCLUDING REMARKS

It is clear that with the advent and development of the tracer method for following carbon in plant metabolism the means for determining the detailed and manifold reactions through which carbon passes into the structure of the plants is at hand. Although some progress in this direction has been made, considerably more time and the efforts of many more laboratories will be required before a clear picture will be obtained of the chemical reactions and interrelations taking place in plants. However, it is not to be expected that this type of work will lead directly to a solution of the unique problems of photosynthesis, namely, the knowledge of the act or acts by which electromagnetic energy is transformed into chemical energy. As a result of these studies with tracer carbon we now believe that the solution of this problem is more likely to be found in investigations of the photochemical production of oxygen by isolated chloroplasts (grana) in the presence of suitable oxidizing agents.

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INFLUENCE OF LIGHT ON PLANT GROWTH[1]

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Introduction

Plants from the simplest unicellular alga to the most complex seed plant respond to sunlight entirely apart from photosynthesis. Some forms, for example the slime molds, usually avoid light but are positively phototactic prior to sporulation. Phototropisms have long been observed and studied in a wide range of plants from the fungus *Pilobolus* to the angiosperm *Avena*. Differentiation of new structures in higher plants is controlled by light, a conspicuous example being regulation of flower bud formation. Light, through periodic duration, also regulates such processes as accumulation of food reserves by cells in the leaf bases of onion during bulb formation (1), changes in total acidity in leaves of certain plants (2), and variation of anthocyanin content of leaves or floral structures (3). Internal anatomy is affected, as in formation of Casparian strips in the stems of various dark-grown seedlings (4) and in changed activity of the cambium (5). Leaf abscission is to some extent controlled by daylength (3), through formation or functioning of an abscission layer. Irradiation may bring about apparently opposite responses in different species. Illumination for only a few minutes in the middle of the daily dark period may, for example, prevent flowering of soybean or promote flowering of barley (6). The photoresponses of plants at different ages or stages of development may be opposite, as was mentioned for the slime mold. Intensity of radiation determines whether phototropic bending is positive or negative.

Photochemical reactions must, of course, play a part in these actions of light. The evidence that such chemical action has occurred usually depends upon an immediate or delayed morphological change which may be as apparently direct as the phototactic migration of a cell of *Chlamydomonas*, or as indirect as the long-day-induced differentiation of flower buds and the elongation of internodes of barley. Less commonly the action is recognized by analysis for some constituent that is destroyed or formed in the reaction. Only in comparatively rare instances, such as photosynthetic action in the presence of chlorophyll, photooxidation of indoleacetic acid sensitized by riboflavin, and transformation of protochlorophyll to chlorophyll, has enough been learned about the sequence of reactions to permit measurements of some reactant.

Although light influences many phases of plant growth and development relatively few photoreactions probably are involved. One of these is responsible for photosynthesis and another for phototropisms, phases of which are discussed in detail in other chapters of this book. A third mechanism

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[1] This review covers the period approximately from 1944 to 1949.

is apparently common to photoperiodic regulation of flowering, formation of storage organs, abscission of leaves, coloration of foliage, and various other morphological changes. This mechanism also probably is responsible for regulation of leaf size and stem length of dark-grown pea seedlings by small amounts of light, and possibly for other responses that are not photoperiodic in the generally accepted meaning of the term. A fourth type of photoreaction probably influences dormancy of seeds, spores, and other structures in some plants. Still other light reactions may exist; however, a great many of the commonly observed changes in growth that are controlled by illumination of plants belong to the second and third types.

This paper is concerned mainly with light responses that are regulated by the third mechanism. It discusses photoreactions controlling flowering in several different plants but does not attempt to cover other aspects of photoperiodism, such as results from grafting, the interactions of various environmental factors with photoperiod, and attempts at extraction and identification of a flower-inducing hormone. It reviews growth responses of etiolated plants that are controlled by the photoreaction operative in the photoperiod response and discusses several other plant responses for which the mechanism of light control has not yet been determined. It also includes a brief discussion of artificial light sources suitable for plant growth.

MORPHOLOGICAL RESPONSES OF PLANTS TO LIGHT

Since the morphological change resulting from photochemical reactions in plants is the usual means of observing the effects of those reactions, the importance of careful and detailed morphological observation is obvious. This is especially true for such a morphologically complex response as flowering, in which the flower bud represents a fairly late stage in a series of morphological events. A few of these changes are therefore described in detail for certain of the plants that have been used most extensively as objects of photoperiodic investigation.

During the last 10 or 15 years increased attention has been given to the photoperiodic conditions that bring about the transition from vegetative to reproductive development as distinct from conditions that influence the subsequent development of flower buds so induced. This has necessitated the very early identification of the changes leading to flowering, which has in most cases been accomplished by dissection of the terminal or axillary buds of the plants. The complexity of the mechanism by which light controls flowering appears so great that many investigators have felt forced to subdivide the process and to attempt to separate these first effects which surely depend upon photoreactions from subsequent ones which may or may not depend on such reactions (7, 8, 9). This procedure, of course, has many obvious advantages but it also has certain pitfalls, some of which can be avoided if adequate knowledge of the morphological changes likely to be involved is at hand.

Two short-day plants that have been used very extensively for investiga-

tions of photoperiodic control of flowering are soybean, *Soja max* var. Biloxi, and cocklebur, *Xanthium saccharatum*. Initiation of flowers in each may result under ideal conditions from a few short photoperiods; two in the case of soybean and one in the case of cocklebur. The microscopic structures induced can be identified by microdissection methods within a week after the treatment is started. Early development of both plants tends to be erect and unbranched. In the axils of their lower leaves buds are present which are vegetative if the plants have been grown under conditions of long photoperiod. Dissection of such a bud from soybean (8) reveals a side axis consisting of several nodes and unelongated internodes and primorida of vegetative leaves. In the axils of these primordial leaves there may be still younger buds which in general repeat the pattern of development of the first. At the next higher node of the main axis is found a repetition of the structures just described, except that usually the axis of such a bud has one or two fewer nodes. This reduction in number of structures in the bud continues rather regularly at each successively higher node until a level is reached at which the bud consists of a meristem, two prophylls, and a single, compound-leaf primordium. The prophylls are small, bract-like leaves that are always differentiated by a newly organized soybean meristem before it gives rise to further structures. Such a reduced bud is located near the top of the main axis in the axil of a leaf primodium. Usually three to five younger leaf primordia occur on the main axis above this level and in their axils there is as yet no evidence of bud formation, except possibly at the lowermost in which a dome-shaped meristem may have begun differentiation of the two prophylls. The vegetative soybean and the vegetative cocklebur are much alike in their basic plans of construction. Plants of either kind that have four to six expanded leaves may have as many as 100 or more primordial leaves already differentiated and many terminal meristems actively producing more. Thus there are countless places on such plants at which further vegetative structures or flowers may be formed, depending upon the photoperiodic conditions to which the plants are subjected.

Despite the fundamental similarity in arrangement of vegetative structures of soybean and cocklebur, there are striking differences in the way they form flower primordia when they are subjected to short photoperiods. If a soybean that has been produced under conditions of long photoperiod is subjected to two to four short photoperiods and is then returned to long photoperiods, it may produce a few flower primordia and then resume the production of vegetative structures (10). Such flower primordia form in the axils of leaves which, at the time the treatment was started, were third or fourth from the tip of the main axis or at a like position with respect to the tip of any side axis. The meristems that give rise to flower buds are thus newly organized terminal meristems that have not yet differentiated the primordia of any vegetative leaves. Such a meristem gives rise to two prophylls and then to one or more bracteal leaves, each with a flower in its axil. The bract is thus the first recognizable step in the differentiation of flowers

and is formed before the bud. The meristem itself remains intact but its cells become vacuolate and stain less heavily than actively dividing meristem cells and it permanently ceases further differentiation.

If the treatment of soybean with short photoperiods is given for only a few days, flower buds may be formed at one or more nodes of the main axis and at one or more similarly located nodes of the various side axes. If the treatment is given for eight or ten days, terminal inflorescences may be induced. In the latter case the terminal meristem of the main axis discontinues the production of primordia of vegetative leaves that are compound, petiolate, stipulate structures, and produces primordia of several bracteal leaves instead. A Biloxi soybean may produce from four to twelve or more of these bracteal leaves with a flower in the axil of each. The terminal meristem then stops differentiating new structures but remains intact and can be identified microscopically at the tip of the inflorescence. The flower-inducing stimulus of a brief treatment is thus of limited effectiveness in soybean and it rapidly and permanently ceases to be effective in the production of further floral organs. If the treatment is of longer duration, its effects are seen first in the axils of very young leaf primorida and last at the apex of the main axis.

With cocklebur the course of development is entirely different. The first recognizable effect occurs at the very tip of the main axis. The terminal meristem begins to enlarge within a few days after the beginning of treatment and it quickly becomes covered with spirally arranged, papilla-like structures which are the primordia of individual flowers and their respective subtending bracts that make up the terminal inflorescence which is always male. If the induction treatment is of more than one day's duration, the meristem appears to proceed directly to the production of such an inflorescence without forming any further primordia of vegetative leaves. If the treatment results from a single dark period, the length of which is near that critical for floral induction, the production of such an inflorescence still may occur but only after the primordia of several additional vegetative leaves are formed. Male inflorescences are also formed by the terminal meristems of many of the axillary buds, but only of those buds in which one or more vegetative leaf primordia have been differentiated prior to the short-day treatment.

Female inflorescences are regularly formed at axillary buds situated just below the terminal male inflorescences, both on the main axis and on side axes. At the time the short-day treatment is begun the terminal meristems of these buds either are not yet visible or exist as slight domes in the axils of the youngest leaf primordia. The male inflorescence of a cocklebur thus arises from a meristem which has formed primordia of several vegetative leaves. The female inflorescence, on the contrary, normally arises from a meristem which has not previously differentiated the primordia of any vegetative structures. Thus in the case of both soybean and cocklebur, meristems that have differentiated one or more primordia of vegetative leaves must follow a clearly defined pattern of development following short-

day treatment, which is different from that followed by newly organized meristems that have not yet differentiated any vegetative structures. Why this should occur is not known, nor is it known why the first flowers are formed at the terminal of the main stem of cocklebur and from newly formed axillary meristems of soybean.

The details of development of the long-day plant *Hyoscyamus niger* as presented by Melchers & Lang (11) are not fundamentally different from those described above for soybean and cocklebur. When subjected to flower-inducing treatments, however, *Hyoscyamus* resembles soybean in that the terminal meristem of the main axis retains its capacity to differentiate new structures for a much longer time than does that of cocklebur.

Some plants must attain a certain stage of development before flowering can occur. For both spring and winter rye, Purvis & Gregory (12) found that the number of leaves formed before flowers were initiated could, under ideal conditions, be as low as seven but not lower. First flowers usually form on Biloxi soybean, according to Borthwick & Parker (8), at node four or five if the plants receive short days as soon as they emerge. This condition, which is somewhat analogous to that in barley, is apparently dependent upon the time required for expansion of first leaves and the rate of differentiation of new nodes by the terminal meristem. As quickly as the first leaves attain adequate size and maturity they are able to exert photoperiodic control over any newly formed terminal meristem that has not yet differentiated primordia of compound leaves. The embryo stem in the dormant seed contains only three nodes but its terminal meristem adds several more during germination. The result is that the first meristem that can be controlled by the leaves after they have become photoperiodically functional is located at node five or sometimes as low as node four.

The condition known as "ripeness to flower" is apparently attained in soybean before completion of germination, for the meristems are responsive to a flower-inducing stimulus as soon as the leaves are sufficiently advanced to transmit such a stimulus to them. It seems probable that the minimum leaf number of seven found in rye by Purvis & Gregory (12) may result from circumstances similar to those described for soybean. Barley seedlings, for example, were found by Borthwick, Parker, & Heinze (13) to have five nodes on the fifth day after the seeds were planted. The first leaf had not emerged from the soil until the fourth day and was still very small. It would be remarkable if at least two more nodes were not added before such a leaf could deliver a flower-inducing stimulus to the terminal meristem.

PHOTOREACTIONS OF PLANTS

Action spectra for photoperiodism.—Photoperiodism was reviewed by Hamner (14) in 1944 and by Murneek & Whyte in 1948 (15). Since these reviews were sufficiently comprehensive, a further detailed review here is unnecessary. With respect to the mechanism of the reaction Murneek (16) points out that " . . . there is little more than circumstantial evidence, fragmentary at that, on the possible steps involved in the reaction of plants

to the photoperiod." Recognition that the process is divisible into several steps, however, constitutes an advance in itself. Perhaps the chief contribution of such theories as have been advanced by Hamner (17), Harder & Bode (18), Lang & Melchers (19), Gregory (20), and others to explain the reaction has been the emphasis they have given to this concept that the process is divisible.

One step in the photoperiodic process that effects control of such plant responses as flowering, bulbing, leaf abscission, or various others, is a photoreaction. Razumov (21) has shown with both long- and short-day plants that red and yellow radiation, when used to extend short natural photoperiods, had the same effect as additional daylight on the photoperiodic reaction, whereas green, blue, or violet radiation was the same as darkness. Katunskij (22) and Kleshnin (23) however, found that radiation from any part of the visible spectrum influenced the photoperiodic reaction. The intensities required varied from one region of the spectrum to another. Katunskij (22) reported that the order of effectiveness of the various spectral regions was red, yellow-orange, blue and green, the latter being least effective. Kleshnin (23) showed the necessity of using low intensities of radiation if differences n effectiveness of different spectral regions were to be distinguished, and suggested that failure of some investigators to recognize this point may account for some of the contradictory results that have been reported.

Since various wavelengths of visible radiation are of different effectiveness in controlling such plant responses to photoperiod, it follows that a pigmented substance of certain characteristics must be present in the plant. Such a substance is necessary to absorb those wavelengths that are effective in the chemical reaction and to transfer the energy to the reacting materials. Not only is the pigment unidentified but nothing is known as to the nature of the reacting substance to which the pigment delivers energy. Fortunately, however, there are experimental methods by which certain characteristics of the unknown pigment may be reliably determined. These involve determining the relative effectiveness of radiation from each part of the spectrum. From such data an action sprectrum curve, relating effectiveness to wave length, can be constructed. Action spectra give information about the pigment, of value for its isolation and identification, and they show very clearly whether or not two apparently unrelated plant responses are controlled by the same photomechanism. Action spectra, in fact, provide an experimental approach by which information dealing with the initial light reaction can be obtained without assumption as to mechanism. This procedure has been used extensively by Parker et al. (10, 24, 25) and Borthwick et al. (26) to study the photoperiodic reaction and to link it with certain other plant responses. That work is described at some length in the following paragraphs.

Determination of the action spectrum for a plant response, such as control of flowering, imposes several requirements. The radiation must be of high intensity and purity in a given wave length region and must cover an area as large as the leaf surface that is to be irradiated. In addition the work

is greatly simplified if the response studied is one that can be induced by relatively brief periods of irradiation in order that there may be time to treat an adequate number of plants in a single experiment.

These workers used a two-prism spectrograph which has been fully described by Parker *et al.* (10). Action spectra for control of flowering were determined for two short-day plants, Biloxi soybean and cocklebur (10) and for two long-day ones, Wintex barley (26) and an annual variety of *Hyoscyamus* (25). Seedlings of these plants were grown in the greenhouse under photoperiodic conditions that were not conducive to flowering. When they had attained an age and size suitable for experimentation they were moved to controlled-environment rooms. Here, during the experimental period, photoperiods of 10 hr. for soybean, 12 hr. for cocklebur and *Hyoscyamus*, and $12\frac{1}{2}$ hr. for barley were applied daily. These photoperiodic conditions induced prompt floral initiation in the two short-day plants and prevented floral initiation for the duration of the experimental period in the two long-day plants. The photoperiodic balance, however, was so delicate that addition of a relatively small amount of radiation during a brief time near the middle of the respective dark periods reversed the response of each plant, preventing floral initiation in the short-day plants and inducing it in the long-day ones. It was at this point in the photoperiodic schedule that radiation from the spectrograph was applied. Each experiment consisted of finding the minimum energy at each of many wavelength stations throughout the visible spectrum that would just bring about this reversal of response. To do this, plants were irradiated at five different levels of energy, each of which was double the immediately preceding one, giving a total energy range for each series of about 16-fold.

These energy variations were made by varying time, it having been found in preliminary experiments with unfiltered radiation from incandescent-filament lamps that reciprocal variations of time and intensity over a time range of 1 min. to more than 2 hr. resulted in no detectable difference in plant response. It was also found by testing with dark-period interruptions of only a few minutes' duration that there was no significant difference in the amount of energy required to produce a given plant response so long as the interruptions were made during a two-hour period which was centered for some kinds of plants 1 hr. after the middle of the dark period and for others 30 min. earlier. If the interruptions were made earlier or later than this time, greater energy was required; accordingly in all experiments with the spectrograph the dark period interruptions were made during the two-hour period of maximum efficiency.

The treatments consisted of one interruption per dark period for three successive dark periods for cocklebur, for six successive dark periods for Biloxi soybean, nine for Wintex barley, and 10 to 18 for different experiments with *Hyoscyamus*. Following treatment, the plants were again subjected to photoperiodic conditions that were not conducive to flowering. After 10 or more days of development the plants were dissected and the presence or absence of flower primordia was recorded.

The intensity of the flower-inducing stimulus influences the rate and extent of flower bud initiation, as has been discussed earlier. A comparatively weak stimulus may result in the very first evidence of flower bud formation, whereas a somewhat stronger one may induce more advanced stages of development of many more buds. It is possible to assign dissected plants to different classes (26), each representing a more advanced stage of bud formation than the last; but it must be kept in mind that these classes give no quantitative information concerning the intensity of the flower-inducing stimulus required for their respective stages of development. It is completely valid, however, to select any particular class as a reference in determining the energy required for a given response.

The action spectra obtained for the four different plants were fundamentally identical. The lowest energy to produce a given response was found with all four plants to occur in the red region of the spectrum between the approximate limits of 6,000 Å. and 6,600 Å. There was a sharp cut-off in the vicinity of 7,200 Å., and in the region from 5,000 to 5,600 Å., with a rapid increase in effectiveness accompanying that in wavelength. The region of minimum effectiveness was at about 4,800 Å. for three of the plants; for *Hyoscyamus*, the fourth, this point was not definitely determined. Effectiveness increased again at wave lengths shorter than 4,800 Å., but the energy requirement for equal response was far greater for this region than for the red.

These results mean that the photoreceptor for the energy used in the photoperiodic control of flowering is the same for long- and short-day plants. There are two strong arguments that this photoreceptor is not chlorophyll. First, in the photosynthetic reaction the efficiencies per unit incident energy in the red and blue are equal but in the photoperiodic reaction the efficiency per unit incident energy is far less in the blue than in the red. Second, the region of maximum effectiveness for photosynthesis in the blue is very close to the region of minimum effectiveness for the photoperiodic reaction. Stronger evidence against chlorophyll appears in the work of Went (27) and Parker *et al.* (24). Went observed that the length of leaves of dark-grown peas was markedly increased and stem length was decreased if the seedlings were briefly exposed to very low intensities of light at certain times during their development. He observed further that radiation from the red end of the spectrum was most effective in causing this response. Parker *et al.* (24) determined the action spectrum curve for the leaf growth response and found that it was fundamentally the same as for the photoperiodic control of flowering. This means that the radiant energy for the reaction that controls leaf growth is absorbed by the same pigment or type of pigment that is operative in the photoperiodic control of flowering. The two plant responses are thus regulated by the same photoreactive mechanism. The dark-grown peas contain little, if any, chlorophyll and although protochlorophyll is present, the amount of radiant energy required to regulate leaf growth is far lower than that required for apparent greening.

Certain races of albino maize and barley respond much like dark-grown seedlings of potentially green strains. When seedlings of genetically pure

green races of barley are grown in the dark the second internode elongates to a length of many millimeters, depending upon the variety. In the light this internode elongates very slightly. Second internodes of albino seedlings grown in the dark attain almost exactly the same lengths as their potentially green counterparts, and when grown in the light they are equally short. Similar observations have been made with maize. In these particular strains the albino seedlings contain no pigmented substances in sufficiently high concentration to give their leaves apparent color. The action spectrum of such albino seedlings has not been determined but it is likely similar to that for the first internode of *Avena* as determined by Weintraub & Price (28) and by Goodwin & Owens (29). These two groups of workers obtained very similar results with *Avena*, and Goodwin & Owens regarded the action as due to protochlorophyll. They attributed the lack of effectiveness in the blue to screening by carotinoids. Since curves (30) for transformation of protochlorophyll to chlorophyll are not in agreement with this, more work, especially with albino plants, is needed to clarify the problem with regard to screening pigments. At this stage protochlorophyll remains a very definite possibility. Parker *et al.* (24) regard these responses of *Avena* as probably due to the mechanism that controls leaf growth in dark-grown peas. The carotinoids and riboflavin would be excluded from consideration as active pigments because they are transparent in the red where the action curve shows that the effective pigment absorbs strongly.

What are the possibilities that the effective pigment could be something other than protochlorophyll? The only guide to its identity is the action spectrum. It can be inferred that the concentration of the pigment is very low, certainly below the limit of visibility in the dark-grown peas and albino seedlings and probably equally low in the green leaves of photoperiodically sensitive plants. It could thus be some substance the presence of which had previously escaped detection in plants of this type. One substance, the absorption spectrum of which would permit it to exhibit such an action spectrum, is phycocyanin. This pigment occurs abundantly in blue-green algae but presumably has never been reported from seed plants.

Phycocyanin, according to Svedburg & Katsurai (31), has a very high absorption in the red just beyond 6,000 Å. and a minimum near 4,800 Å. It has another maximum in the visible near 3,800 Å. and one in the ultraviolet near 2,800 Å., these two, however, being far lower than the absorption in the red end of the spectrum. Phycocyanin is transparent at wave lengths longer than 7,000 Å. The absorption requirements of the pigment that is responsible for the action curves for the photoperiodic control of flowering and for leaf-length increase in dark-grown peas, are thus quantitatively met by the absorption characteristics of phycocyanin.

Phycocyanin need not be the effective pigment. Proteins having other noncyclized tetrapyrole chromophoric groups in which the double bonds are completely conjugate would also be characterized by an absorption spectrum similar to that of phycocyanin. The chromophoric group could, for instance, be either glaucobilin or pterobilin and might have substituent groups making

it different from either of these. If conjugation of double bonds were in-complete, the absorption maximum would be shifted too far into the yellow-green portion of the spectrum, as in the bile pigments.

Physiological responses.—Little can be inferred from the data as to the nature of the physiological process upon which the photoperiodic pigment acts in the cell. The fact that such dissimilar responses as control of flowering and regulation of leaf size are governed by the same basic light reaction suggests that the effect of light might be to modify some general physiological characteristic of the cell. Such a possibility is open to experimental verifica-tion and certain relevant data are already in existence. Stålfelt (32) and Virgin (33) found that the viscosity of the cytoplasm of *Elodea* leaves was changed by light energies as low as 10 foot-candles applied for 15 sec. The changed viscosity persisted for several hours in the period of darkness following a 15-sec. treatment. Priestley's observations made many years ago (4) on dark-grown peas are also of interest in this connection. He found that a 17 per cent cane sugar solution would not plasmolyze the cortical cells in the plumular hook region if the peas had been grown in the dark, but it plasmolyzed them readily if such dark-grown plants had been irradiated 2 min. on each of three consecutive days prior to observation.

Another phenomenon requiring a very low amount of energy was re-ported by Pearsall & Bengry (34) for growth of *Chlorella*. They grew *Chlorella* in several different light intensities and in the dark in a complete nutrient solution to which glucose was added. Marked increase in rate of growth resulted from the application of light and an intensity of less than two foot-candles was more than sufficient to saturate the reaction.

Action spectra for these various responses have not been determined, so it is not known whether the light reaction regulating them is the same as that for controlling flowering. Energy requirements are of about the same magnitude, however, and it seems probable that some, at least, are controlled by the same basic mechanism.

An action spectrum for the growth of pea stems has not been determined, but Went (27) found that inhibition of length growth was much greater in red light than in an equal intensity of blue. This suggests that the same pigment concerned in growth of leaves of etiolated peas may also be active in controlling stem length. Galston & Hand (35) investigated this light response of dark-grown pea stems in an effort to find a biochemical explana-tion for the light effect. They cultured isolated stem segments in solutions of .1 to 1.0 μg. per cc. of indoleacetic acid (IAA). In the dark these seg-ments made maximal elongation, but when such segments were exposed to visible radiation their growth was inhibited regardless of the concentration of IAA. Avena tests for auxin concentration indicated that the decreased growth response in light was not due to a lowered auxin concentration within the tissues, and further tests confirmed the fact that photoinactivation of auxin was not responsible for the light-induced inhibition of growth. Exami-nation of the culture solution showed that the IAA in control flasks without sections remained constant, while the IAA in the solutions with sections dis-

appeared almost twice as fast when irradiated as when kept in the dark. Galston & Hand (35) suggest that a nonauxin system is responsible for the light-induced growth inhibition. In a later work Galston (36) showed that riboflavin could sensitize the *in vitro* photooxidation of IAA. Galston & Baker (37) have extended the work with riboflavin and have found that when riboflavin is added to the culture solution in the dark, concentrations of 0.1 and 1.0 μg. per cc. stimulated growth. These same concentrations of riboflavin were extremely inhibitory to growth when the segments were irradiated, but the decomposition products of riboflavin, i.e., lumiflavin and lumichrome, were not inhibitory. The action spectrum for the *in vitro* destruction of IAA in the presence of riboflavin followed the absorption spectrum for riboflavin, and was characterized by a maximum at 4,400 Å. and a long wave length cut-off at about 5,200 Å. In experiments with concentrated brei from dark-grown peas a similar action spectrum was obtained. Therefore, Galston & Baker concluded that riboflavin sensitized the photo-inactivation of auxin or some other growth factor thereby exerting an inhibitory effect on growth. Although this reaction is effective in controlling growth of stem segments it cannot be the principal one that controls stem length in the intact plant, for Went (27) has shown that red light is more effective than blue for this response.

Cajlachjan (38) several years ago proposed that a special hormone, "florigen," controls flowering in plants. His suggestion has been accepted by many workers and extensive studies have been made concerning conditions under which it is formed and factors that control its translocation through the plant and across graft unions (39, 40). Despite the efforts of many investigators to isolate and identify it, florigen still remains unknown.

Experimental data nevertheless show that flowering and other photoperiodic phenomena are probably controlled by chemical substances produced in the plant, possibly of a hormone nature. It is not at all clear whether each kind of plant response is controlled by the same substance or by different ones, nor is there any evidence showing just how light affects the chain of events involved in the formation and functioning of such materials. For this latter reason further discussion of regulatory materials produced in plants is not included.

RADIATION FOR PLANT GROWTH

The use of controlled-environment rooms in plant physiology research has become increasingly important because of the necessity in many kinds of experiments of being able to grow comparable plants at any season of the year. The greatest limitation of such rooms is the source of artificial visible radiation, and as yet no completely satisfactory source has been developed. In general two main types of lamps are being used, the alternating current carbon-arc and the fluorescent.

Parker & Borthwick (41) have reported on their controlled environment rooms, which have, as the principal source of artificial radiation, alternating current carbon-arc lamps. The lamps burn "Sunshine" carbons that are

cored with rare earth metals of the cerium group which greatly increase the energy in the visible spectrum, particularly in the blue-violet end. The radiant energy from these lamps is derived principally from the flame. With this type of arc a part of the carbon can be replaced with chemical compounds capable of radiating efficiently when in a highly heated gaseous form. These compounds vaporize with the carbon and diffuse throughout the flame of the arc, making it luminescent. Since the whole flame is made luminous, the radiation source is one of large area and the spectral distribution can be altered by introducing different chemicals into the coring material. Radiation from such carbons does not duplicate solar radiation, but reasonably satisfactory growth of soybeans can be obtained with this as the only source of radiant energy. Soybeans grown with radiation emitted by "Sunshine" carbons alone have repeatedly been found to have a lower carbohydrate content than soybeans grown with solar radiation. By supplementing the radiation from the arc with that from incandescent-filament lamps more red radiation was provided. Soybeans grown under this combined source of radiation had sturdier stems, more dry weight, and greater carbohydrate accumulation. The radiation from experimental carbons cored to simulate the spectral distribution of the combined arc and incandescent-filament source failed to produce as much dry weight, protein, or carbohydrate as when the arc was supplemented with incandescent radiation.

Withrow & Withrow (42) have compared the radiation from incandescent-filament, fluorescent, and mercury-arc sources on the growth of aster, spinach, soybean, and tomato. On an equal power consumption basis, aster produced more dry weight with incandescent radiation and spinach produced more with fluorescent. Plants grown with mercury-arc source were very poor. In another series of experiments in which equal radiant energies were maintained, Withrow & Withrow compared the growth of spinach, soybean, and tomato when grown with fluorescent lamps and with incandescent and mercury-arc combined in three different proportions. They report the greatest production of dry matter for all species under the incandescent plus low-mercury combination. The authors point out that the mercury-arc lamp has a very low efficiency for plant growth, due to the fact that nearly two-thirds of the total visible radiant energy of the H-1 mercury-arc does not coincide with chlorophyll absorption bands. In general, Withrow & Withrow report that greatest dry weight production and height occurred with incandescent sources, when compared on the basis of equal total electrical power consumption per plant, equivalent foot-candles, or equal total energies in the visible. However, the soybeans grown by them with incandescent radiation were taller and contained less dry weight than somewhat comparable plants that were grown by Parker & Borthwick (41) with radiation from the carbon-arc lamp supplemented with incandescent.

Despite the merits of the carbon-arc lamp as an exceptionally good source of radiation for plant growth chambers, the majority of recent installations have been made with fluorescent lamps. Many investigators (41, 43, 44, 45) have reported details of construction of such installations but, almost with-

out exception, they have not published growth data which might be used for comparison of the various rooms. Therefore, there are very few data available as to plant growth with fluorescent sources. Parker & Borthwick (41) have compared the growth of Biloxi soybeans when grown in sand culture for 21 days with three different sources of radiant energy. One source was a panel of 18 eight-foot slimline fluorescent lamps with and without the radiation from filament lamps; another was a carbon-arc lamp burning "Sunshine" carbons supplemented with incandescent-filament lamps; and the third was solar radiation as received in the greenhouse. Cultural conditions were identical under all three sources. Temperature was maintained at 80°F. during the 16-hour photoperiod and at 70°F. during the dark period for plants grown in the two artificial sources of radiation. Temperature in the greenhouse was maintained near 70°F. in the winter, but was not controlled at other times.

The fresh and dry weights of the plants were greater and the stem length was less when the radiation from the 4,500° white fluorescent lamps was supplemented with that from the incandescent-filament lamps. Plants grown with radiation from carbon-arc lamps supplemented with incandescent lamps were always shorter and heavier than those grown with radiation from fluorescent lamps. The leaf area of the plants grown with radiation from the arc lamp was obviously greater than that of plants grown with the radiation from fluorescent lamps, and the stems of the plants under the former conditions were very much sturdier than those grown under the latter. Soybeans grown in the greenhouse during the fall and winter months were not as heavy as the plants grown with radiation from the fluorescent lamps and were usually much shorter. However, during the spring months the plants grown under these two conditions were very similar, as judged by stem length and weight.

Although one commonly thinks in terms of the light requirements for photosynthesis when considering artificial sources of radiation, it is important to keep in mind that innumerable light-controlled responses are occurring simultaneously in the plant. Thus, such a process as photoperiodic regulation of flowering has different spectral sensitivities from those of the photosynthetic reaction. Responses depending on the phototropic mechanism have still different spectral requirements from either of these. When one considers that the spectral requirements of each of these three kinds of reaction, and probably of others, must be met simultaneously in an artificial light source, the difficulties become more apparent. Therefore, it becomes increasingly obvious that any source of visible radiation for plant growth must contain ample blue and red for these controlling processes and that a proper balance of such wave lengths and possibly others is important to the formative growth of a plant.

The application of supplementary light in greenhouses has become more common during the last decade. Generally such applications have been for the purpose of controlling the type of morphological development in photoperiodically sensitive plants. For such purposes the incandescent-filament

lamp is well suited because red is the effective region of the spectrum for control of these responses and much of the visible radiation from this lamp is in the red. With the advent of fluorescent lamps, a few installations have been made in the greenhouse in an effort to supply additional radiant energy for assimilation, particularly during periods of dull, cloudy weather. However, caution should be observed in using this type of radiation because a few instances have been reported in which plants have not responded in a normal manner when it was used to supplement daylight.

Neon lamps have been used in various European greenhouses to supplement solar radiation. Meurman (46) reports that under Finnish conditions it is profitable to use such radiation for the production of early greenhouse tomatoes. In these experiments the lamps were used for eight hours per night for 30 and 60 nights. The plants that were irradiated were much larger and they flowered and matured fruit earlier than the controls.

Roodenburg (47) in the Netherlands, has tested most of the commercial light sources, such as neon tubes, incandescent-filament lamps, mercury-vapor arc, and fluorescent lamps for their applicability as sources of radiation for plant growth. He concludes that it is impossible to designate one definite light source as being the most suitable. The choice depends upon the crop and the effect desired. He listed the following four purposes for which artificial light is generally used and recommended a source and intensity for each: (a) stimulation of carbon dioxide assimilation: 50 to 100 watts per square meter of neon light during the night as a supplement to solar radiation, a type of supplemental radiation which has been useful for raising seedlings; (b) extending the length of day: 5 to 50 watts per square meter of incandescent-filament lamp radiation, the intensity depending on the species; (c) forcing plants with infrared: 100 watts per square meter of incandescent-filament lamp radiation, under which conditions tulips can be forced without daylight; and (d) elimination of blue light deficiency: slight addition of radiation from a mercury-vapor lamp, of importance when plants are cultured without daylight. Roodenburg (47) also tested the radiation from fluorescent lamps for plant growth in the greenhouse. While the radiation had a good spectral distribution, so many lamps were required to produce adequate intensity that they intercepted more natural radiation than they contributed. For studies of germination or early seedling development and for areas of limited size, satisfactory results were obtained with fluorescent lamps as the sole source of radiation.

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PLANT TROPISMS[1]

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INTRODUCTION

Responses of plants or plant organs in the form of "growth movements" to oriented stimuli are characteristically designated as tropisms. These growth movements are brought about by unequal elongation rates on opposite sides of the organ so that curvature results. The fundamental distinguishing feature of tropisms is the space distribution of the energy of stimulation, which must be arranged to give an energy gradient across the longitudinal axis of the tissue. Ordinarily, a change in the intensity of the energy input into the organism is the primary characteristic of the stimulus, but in tropisms the emphasis is on responses to changes in the space distribution, without necessarily changing the intensity, of the energy of stimulation. Responses resulting from energy changes, which affect the plant uniformly, are not naturally included in the category of tropisms, but such experiments sometimes yield valuable information to help explain the tropism mechanisms.

Extensive investigations (6, 56) have led to the widely accepted conclusion that the growth responses of tropisms are dependent on the auxins; to a large extent, a redistribution of them. It follows that the ultimate link in the complete understanding of tropisms is the mechanism by which auxins influence growth. Because the status of the relationship between auxins and growth has recently been reviewed by Thimann (53) and earlier by van Overbeek (32), Bonner & Wildman (4), and by Went (57), this aspect of the problem will not be emphasized here. Preceding this final step, is the mechanism which is responsible for the unequal distribution of the auxins, which can be treated primarily as a phenomenon of correlation. The present paper will be concerned with some of the publications which have appeared since previous reviews and will tend to emphasize the correlation aspect of tropisms. An attempt will be made to group the papers according to the type of energy that was used in stimulation, though in frequent instances there will be overlapping.

PHOTOTROPISM

Eleven years ago van Overbeek (31) summarized the status of phototropisms. At that time it could be said that phototropic curvature is considered to be due to different elongation rates, which are auxin controlled, of opposite sides of unilaterally exposed organs. In the higher plants this differential growth is achieved in one of several ways, or possibly a combination of them, depending on the plant and the intensity of illumination. First, the lighted side of the exposed plant responds less to auxin than the shaded side, and it is thought that photo-inactivation of auxin is responsible for the decreased response. Secondly, it has been shown that auxin is trans-

[1] This review covers the period approximately from 1939 to 1949.

ported to the shaded side of some plants when they are subjected to unilateral illumination (Cholodny-Went theory). A third possibility should now be added to these considerations and included in the present discussion, especially in phototropic responses of roots. Increased auxin production resulting from stimulation by light has been suggested to account for negative bending in some instances.

The first step in the phototropic response of plants is the absorption of the radiant energy. In so far as the Avena coleoptile is concerned, there is evidence that carotinoid pigments are responsible for the absorption of the light (10, 55). That the absorption curves of the carotinoids from etiolated Avena coleoptiles correspond to the spectral sensitivity curve of phototropic bending of the seedling was reported by Johnston (22) as well as others. According to Bottelier (5), protoplasmic streaming is quantitatively related to the amount of radiation, and the spectral sensitivity curve of streaming is rather similar to absorption curves of carotinoid pigments of oat seedlings. In an attempt to supply one of the intermediate links for the Cholodny-Went theory of tropisms, Showacre & duBuy (47) have incorporated these observations to postulate a mechanism to account for the unequal distribution of auxins. They suggest that respiration serves as the linkage between the light absorption and streaming with the result that streaming is inhibited more effectively on the illuminated side so that a larger portion of auxin is transported from the apical cells to the shaded side. This theory quite logically explains positive phototropisms, but it is not immediately apparent how it could be used to account for negative tropism of the same organism.

During the last year attention has been directed to an additional light absorbing compound which could be functional in tropic responses to light. While studying the effect of light on etiolated peas, Galston & Hand (19) observed that small amounts of riboflavin in the medium caused a marked inhibition of growth when the tissue was exposed to light. Such inhibition did not occur in the absence of light. Galston (18) subsequently reported *in vitro* experiments, which showed that riboflavin can sensitize the photooxidation of indoleacetic acid, as well as other indole-containing compounds (20). The inactivation of indoleacetic acid apparently is a first-order reaction which requires oxygen. Since the absorption maximum of riboflavin (about 460 mμ) corresponds rather well with the action spectrum for phototropism, Galston suggested that riboflavin could be a receptor pigment in the phototropic responses of plants. This possibility remains to be critically tested.

The importance of the role of destruction of auxin by light in phototropisms has been stressed in a number of instances (57). Recently Kögl and co-workers (23, 24), while studying the absorption spectrum of auxin-α-lactone, found that this auxin lost about 80 per cent of its growth-inducing ability during illumination for measurements. They observed a definite absorption band at 495 mμ, and concluded that the auxin-α-lactone was transformed into another substance with unmeasurable rapidity which in turn gave the characteristic absorption spectrum. The inactive product was named lumi-auxin-α-lactone and later abbreviated to lumi-auxon. Studies

have also been made of the inactivation of auxin-α-lactone by light in the presence of carotinoids with the result that β-carotene was found to be the most effective in inactivation of this lactone from 405 to 494 mμ, which is in the region of its maximum absorption (24). This can be interpreted to indicate that the specific properties of the photo-sensitizer might to some extent limit the significance of the role of photodestruction of auxin in a given phototropic response.

Stewart & Went (52) have studied the effect of light on free moving and bound auxin in the Avena coleoptile. Their results indicate that the amount of auxin moving through an Avena coleoptile section is not affected by light. However, ether extractable auxin was found to be partially inactivated, even by short exposures to white light. Because the free moving auxin is apparently light stable, it follows that it is the bound auxin that is inactivated by the light. Stewart and Went maintain that the auxin is inactivated while it is in combination with some cell constituent (bound form) and that their experiments do not support the idea that the inactivation by light is directly by way of the lactone.

After an extensive discussion of the literature on photo-inactivation of auxin, Oppenoorth (30) set out to evaluate the importance of the role of such inactivation on the over-all phenomenon of phototropic responses of the Avena coleoptile. His data show that exposures up to 150 ergs per sq. cm., using monochromatic blue light (4,360 Å), gave no detectable inactivation of auxin. It follows that the phototropic curvature in this region of illumination must be mediated by other mechanisms. The first positive curvature reaches its maximum when the applied light energy exceeds 330 ergs per sq. cm. Oppenoorth found that photo-inactivation of auxin occurs only in this narrow region, but he maintained that it could not have a role in the curving process, because the inactivation was practically equal on the two sides of the coleoptile. Increasing the light from 700 to 1,400 ergs per sq. cm. resulted in a decreased phototropic response and again had no effect on the inactivation of auxin. The first negative curvature was obtained when the quantity of light was increased to 3,000 ergs per sq. cm., for which no photo-inactivation of auxin was found. Finally, an exposure of 30,000 ergs per sq. cm. was made. Since this is the region of the beginning of the second positive bending, no curvature was observed, but inactivation of auxin was apparent. These data support the conclusion that photo-inactivation of auxin is relatively unimportant in the phototropic responses of the Avena coleoptile.

Additional experiments have been reported to test the Blaauw theory of phototropism in lower plants. Dassek (14) found that rhizoids of *Lunularia cruciata* were phototropically sensitive to illumination by wave lengths from 470 to 497 mμ. The threshold of spectral sensitivity was shifted to 497 to 524 mμ when sunlight was used as the source of illumination. Dassek's data show that these rhizoids manifest a negative phototropism in air, none or slightly negative when submerged in water, and a positive bending in liquid paraffin. When illuminated uniformly, the rhizoids of *Lunularia* exhibit a negative growth reaction. In air, the surface of the transparent rhizoid

apparently serves as a convex lens, which converges the light on the back side. The increased illumination on the back side inhibits growth more strongly there, so that the resultant bending is away from the light. The absence of phototropic bending of the rhizoid suspended in water is presumably dependent on the fact that the difference between the indices of refraction of the water and the cell sap is so small that there is only a slight convergence of light on the back side such that the difference in illumination of the two sides is not great enough to induce curvature. In liquid paraffin the rhizoid functions as a concave lens, which causes the back side to be illuminated weaker than the front side and results in positive bending. It is a matter of conjecture whether auxin transport is involved in these responses or whether the light exerts its inhibitive effect by destruction of the growth-inducing substances.

Data continue to accumulate, from different experimental material, which indicate that lateral redistribution of auxins is certainly an important contributing link in many of the phototropic phenomena. Wilden (59) has reported an examination of the auxin distribution in the Avena coleoptile during positive and negative phototropic curvature. Coleoptiles illuminated so as to give the first positive curvature show an auxin distribution of 17 per cent on the lighted side compared to 83 per cent on the shaded side, 130 to 140 min. after stimulation. The auxin distribution was reversed in the first negative curvature with a ratio of 62 to 38 per cent. During the second positive curvature the distribution was found to be 36 per cent and 64 per cent with the orientation the same as in the first positive stage. Lateral auxin distributions of this magnitude correspond to about 50, 15, and 20 degrees of curvature, respectively. These findings are in general agreement with the previous results of Asana (2). Similar results were obtained by Yamanae (61), using Went's agar diffusion method and the Avena test, from phototropically orienting leaves of *Fatsia japonica*. In the positively phototropic leaves (positive or negative bending being dependent on the age of the leaves) more auxin was found on the shaded side (60:40), while in the negative leaves more auxin was found on the lighted side of the leaf (30:70). It is not clear from the reports of these experiments on lateral distribution of auxins, to what extent, if any, photo-inactivation of the growth-inducing substance is a complicating factor.

A photoelectrical correlation mechanism to serve as the orienting force for redistribution of auxins in phototropisms has apparently never received much emphasis. Schrank (40) has published results of preliminary experiments which show that continuous unilateral illumination of the Avena coleoptile with a light intensity of 16 foot candles at the plant position induces a transverse electrical polarity in the apical region. The shaded side, which will be the convex side, becomes electropositive to the lighted side before bending toward the light starts, but this effect has been observed only for the most apical cells. These observations appear contradictory to the results obtained by Oppenoorth (30), who found that unilateral illumination of the coleoptile by unfiltered mercury light caused the lighted side to

become electropositive to the shaded side. This polarity was obtained by subtracting the longitudinal electrical changes of the shaded side from the simultaneous changes of the lighted side. A common basal contact was used in measuring these electrical changes. Much additional work needs to be done in this area with special emphasis on the effects of intensity and wave length of the applied illumination.

Changes in the rate of synthesis of auxin, which are induced by light, should necessarily be included as a part of the phototropic reaction. Oppenoorth (30) found an increase in the auxin content of the coleoptile tip in experiments in which different light quantities were used. This increase in auxin content of the tip was considered to be due to an increase in its synthesis. Oppenoorth's experiments indicate that the rate of the increase of the auxin synthesis depends on the quantity of light administered per unit time. Similarly the experiments of Naundorf (29) demonstrate that unilateral illumination establishes a higher auxin content on the lighted than on the shaded side of roots of *Helianthus annuus*. Parallel experiments indicated that illuminated roots actually produce more growth substances than non-illuminated, and that the auxin production increases with increased illumination time. In comparison to other light, blue light seems to be most effective both in inducing bending and auxin production on the illuminated side. The negative tropism of *Helianthus* roots is explained by the fact that the light causes an increased auxin production on the lighted side of the apex, thus causing the organ to bend away from the light. In this connection it should be recalled that Langham (25) reported that *Euchlaena mexicana* (along with several other plants) became prostrate in the greenhouse, but when these plants were placed in the dark room they would become erect again. Field and laboratory experiments confirmed the conclusion that such plants are prostrate under field conditions, because they are probably negatively phototrophic to intense light. Although this investigator did not make the suggestion, it is entirely possible that increased auxin synthesis on the illuminated side of the plant could account for this phenomenon.

Some investigators continue to maintain that light-turgor reactions are intimately associated with the phototropism mechanism, especially with the auxin-growth linkage. Some studies along this line are relevant. Earlier experiments by Brauner & Brauner (8), which indicated that the light-turgor reactions of the pulvinus of *Robinia pseudacacia* were brought about by changes in the permeability of the sensitive cells, led Brauner to investigate the light-turgor reactions of the pulvinus of *Phaseolus multiflorus* as well (9). Here, as in the previous study, he found that the responses of the pulvini were dependent on the wave length of the light, intensity of illumination, and on whether the material was submerged in water or kept in air. Monochromatic green light apparently is phototropically inactive, while blue light is more effective than white light of the same energy level in bringing about positive reactions of the pulvini in air. It seems that chlorophyll is responsible for the absorption of light in the red and yellow end of the spectrum, carotene absorbs the blue light, and the ineffectiveness of the monochromatic

green light is accounted for by the presence of an absorption gap around 550 mμ. The relationship of this work to tropic responses involving growth curvature is somewhat indirect; nevertheless, it has promise of revealing significant information.

In a general overview of the phototropism mechanism it seems logical, as Oppenoorth (30) and possibly others have suggested, to assign a triplicate role to auxin. The constituent parts are: (a) photo-inactivation of auxin, which appears to have a less significant part in the phototropic reaction than was originally assumed, over which there is disagreement as to whether the lactone or bound form is inactivated by light; (b) lateral transport of auxin, which still remains as one of the more important links in the chain of events and for which the elucidation of the fundamentally important polar forces necessary to account for lateral auxin transport is still limited and nebulous, although postulations have been presented; and (c) modifications in the synthesis of auxin instigated by illumination. It is rather obvious that any one of these changes could operate singly or in combination with the others. In all events, an unequal distribution of auxin results. To make this formulation complete, only the elusive explanation as to precisely how auxin influences growth needs to be added.

GEOTROPISM

It is generally accepted that plants respond geotropically by utilizing auxins which have been unequally distributed. Apparently the simple re-distribution of auxins, without destruction or change in the rate of produc-tion, is one of the essential links between stimulation and geotropic bending (35). As Went (58) has carefully emphasized, in correlation phenomena such as geotropism, the concern is primarily with the free moving or diffusible fraction of auxin. So far the physical causes or mechanisms of the auxin transport are not well understood, though several theories have been sug-gested. Because there seemed to be a close relationship between the presence of starch and the geo-reception, the statolith theory was formulated. An-other concept has been that geotropic stimulation results in a difference in potential across the organ which then initiates the chain of events leading to geotropic curvature. The investigations of Bottelier (5) seem to indicate that protoplasmic streaming, as an auxin transporting mechanism, functions as part of this chain.

In recent years several interesting responses to geotropic stimulation, which are dependent on unequal auxin distribution, have been observed. Van Overbeek & Cruzado (34) found that a minimum of three days of geo-tropic stimulation (plants were kept in a horizontal position) was required to induce flower formation in the vegetative plants of the Cabezona variety of pineapple. These plants also responded by bending upward. Similar geo-tropic flower induction was not observed in the Red Spanish and Smooth Cayenne varieties. It has been shown that relatively small quantities of auxin will stimulate flower formation in the pineapple plant (33) and that geotropic stimulation generally causes a redistribution of auxins in the plant

(56). Van Overbeek & Cruzado, therefore, explained their observations by postulating that geotropic stimulation causes the auxin content on the lower side of the plant to increase to the extent that the lower portion of the apex starts flower formation. Thus, the auxin production is presumably increased, which in turn stimulates the entire apex to change into the floral stage.

The Cholodny-Went theory of geotropic reaction has been extended, in part, to include responses of leaves. Arslan (1) maintains that the first step of the geotropic reaction of the pulvinus of *Phaseolus vulgaris* is the accumulation of auxin in the lower half of the organ, and that the course of the geotropic reaction is determined chiefly by a change of the suction potentials in opposite halves of the pulvinus. Zhdanova (62) has demonstrated, by decapitation and auxin application experiments, that the degree of geotropic curvature of *Hydrangea* leaves is dependent on the anatomical dorsal-ventral polarity and on the auxin content. His experiments, however, do not attempt to show a difference of auxin distribution in the upper and lower sides of the leaf. A similar explanation has also been presented by Rose (36) to account for the experimental inversion of the geotropic response of the tap root of peas by injection of pure olive oil or commercial peanut oil. It was assumed, though not proved, that the alterations in geotropic responses were caused by the presence of small quantities of auxin in the oils.

Another method of attacking the geotropism problem has been by the use of centrifugal force. In the older work involving geotropic stimulation wide variations in angles of deviation (25° to 65°) were observed for roots subjected to a constant centrifugal force of 1 *g* on a horizontal wheel (48). Small (48), using improved methods, made a series of studies on *Vicia faba* radicles covering a range of angles from 15° to 90°. He maintained that the direction assumed by the root was not the same as the diagonal of forces resulting from gravity and centrifugal force, and that the response of the radicle to gravity was not necessarily the same as its response to centrifugal force. This conclusion has not received supporting evidence. Chance & Smith (12), using seedlings of buckwheat (*Fagopyrum esculentum*), found variations in upward curvature from 38° to 52° when the seedlings were kept in the horizontal position and subjected to 1 *g*. The average curvature was 44.93°. When the plants were placed in the vertical position and rotated to get 1 *g*, an average curvature of 46.06° downward was obtained. Their average curvatures show that buckwheat plants respond predominately to gravity when stimulated by 0.96 *g*, and that they respond predominately to centrifugal force when 1.04 *g* are applied along with the geotropic stimulus. (These authors did not indicate whether or not their data were statistically treated.) Chance & Smith further found that a curvature balance could be obtained between phototropic and geotropic stimulation, and that the tropic influence of gravity added to its equivalent of light stimulation could be balanced by a centrifugal force of 2 *g*. Burkholder (11) has shown that geotropic curvature of the Avena coleoptile can be reversed by continuous illumination from an 85 watt mercury lamp two meters away. There is a differ-

ence in the reaction time for phototropism and geotropism in these compensation experiments, and it also appears that there are changes in the sensitivity of the tissue during stimulation. However, these results and those of Chance & Smith can be taken to substantiate the hypothesis that the stimuli of light and gravity are additive and that their combined effects can be balanced by centrifugal force. These observations are not at all unusual when it is recalled that auxin has been established as a common denominator in all of these responses.

Brain (7) studied the effect of prolonged rotation of hypocotyls of *Lupinus albus* on a horizontal klinostat and found that geotropic responses to 20 min. stimulation were not affected by the previous growth on the klinostat, but he observed that more auxins diffused out of the plants which were grown on the klinostat than out of upright plants. It seems that some explanation as to why the increased auxin content did not result in more extensive geotropic curvature should have been presented in order to clarify Brain's contention that these results, which are consistent with earlier observations, substantiate the idea that a differential auxin distribution is a required link in geotropic responses.

The effect of gravity on the inherent electrical pattern of plant tissue is another response that warrants consideration, because it has been suggested that these electrical fields may serve as the fundamental correlation mechanism in the various tropic responses. Schrank (38, 41) has shown that the entire electrical pattern of the Avena coleoptile changes in a definite manner when the plant is rotated from the vertical to the horizontal position. These electrical changes, which are dependent on living cells (not in agreement with earlier observations), can be detected long before upward curvature starts. If only the transverse component of the electrical pattern is considered for the moment, the data show that the under side of the coleoptile starts to become positive to the upper side within one minute after it is placed in the horizontal position (10 to 20 min. are required for the maximum polarity of about 10 mv. to be established). The positive side is the one that later becomes the convex side. On the basis of the measured rate of auxin transport in the coleoptile cells, it has been calculated that at least six minutes would be required for an auxin molecule to be transported a distance of 1 mm. from the upper to the lower side of the plant (38). These results are taken to indicate that the changes in the inherent electrical polarities are antecedent events in the process of geotropic curvature, and that the auxins are transported to the electropositive side of the plant.

In a series of preliminary experiments Wilks & Lund (60) checked the effect of 0.50 microampere of direct current flowing along the longitudinal axis of the Avena coleoptile for a 30 min. period applied simultaneously with geotropic stimulation. They found that when the current flowed from the apical end toward the base of the plant, there was an inhibition of geotropic bending. No change in bending rate was observed when the same current was made to flow in the opposite direction. It is known from subsequent work that longitudinally applied current also affects the inherent electrical

pattern of the Avena coleoptile (45). While these experiments do not prove that the inherent electrical fields function as the oriented force which is required by the Cholodny-Went theory, they certainly substantiate the possibility.

PLAGIOTROPISM

Apparently only two aspects of plagiotropisms have received recent attention. Snow (49, 50, 51) has reported some experiments concerned with the relationship between plagiotropism and correlative inhibition and others which were designed to test Sach's theory of plagiotropism. This investigator found that hetero-auxin paste, placed on the decapitated apical end of the main stem of *Impatiens roylei*, would keep the lateral shoots epinastic much the same as the intact apex (49). Removal of the developing leaves from the plagiotropic laterals caused them to become more orthotropic. These experimentally induced orthotropic laterals are inhibited by the principal apex much like inherently orthotropic lateral shoots in various other plants. Snow maintains that these experiments tentatively support the theory that epinastism of intact laterals is induced by auxins derived from the apex of the stem, similar to correlative inhibition, and suggests that the two responses are alternative reactions to a common transmissible factor. Apparently there is no clear cut indication as to when epinastism diminishes and inhibition develops. In later experiments (50), Snow observed that "correlative inhibition" could not move into the plagiotropic branches of *I. roylei* and other plants, and that hetero-auxin in lanoline paste, applied either to the cut end of the decapitated branch or to the decapitated main stem, could take the place of developing leaves in keeping the lateral branches epinastic. Because of these results, the previous tentative conclusions had to be modified. Since these last experiments show that "inhibition" does not more upward in the plagiotropic branch, it seems probable that two different transmissible influences are involved. Therefore, Snow suggests the possibility that the induction of plagiotropism (via epinastism) and inhibition are indirect responses, both being initiated by the production of auxin in the main stem apex. It appears, then, that while the lateral branches have developing leaves attached, they have adequate auxin, and the inhibiting factor can not move up into them. Under these conditions the agent inducing epinastism exerts its effect. When the lateral branches are without leaves, or source of auxin, inhibition takes the predominant role, and the epinastism-inducing influence is not effective.

According to Sach's theory of plagiotropism of laminae, the direction in which a part of a leaf bends depends on whether the attached end is toward or away from the direction of the stimulus. To test this idea, Snow (51) cut tongue-shaped flaps along the median axis of young leaves of *Stachys silvatica*. The flaps were cut so that the attached end was in the vicinity of the tip of the leaf. When subjected to phototropic stimulation, the flaps generally curve in the same way as the rest of the leaf. According to Sach's theory, the flap and the remainder of the leaf should have curved in opposite direc-

tions, because the point of attachment of the flap to the leaf was at the opposite end of the point of attachment of the leaf.

In this informative work on plagiotropisms the emphasis was placed on secondary and intermediate mechanisms, which are directly dependent on chemical agents. At this point it must again be stressed that the crux of this problem lies in the elucidation of orientated forces which can account for the redistribution or unequal activity of the auxins or "influences" which are responsible for inhibition or epinastism.

Thigmotropism

Tropic responses to mechanical stimulation, including the twining phenomena, have received limited attention during recent years. In agreement with earlier observations, the experiments of Schrank (37) have shown that mechanical stimulation of the apical 10 mm. of one side of the Avena coleoptile induces bending toward the stimulated side. Such stimulation also establishes a transverse electrical polarity preceding the curvature response. The stimulated side of the seedling becomes electronegative to the nonstimulated side. It was noted that the subsequent bending was toward the negative side of the plant, which is the same as it was for geotropic stimulation. Further experiments, when the under side of the oat seedling in the horizontal position was stimulated mechanically at 5 min. intervals, disclosed two additional facts: (a) the under side remains electronegative to the upper side, which is opposite to the orientation of the electrical polarity induced by gravity as the only stimulus; and (b) such mechanical stimulation prevents the plant from curving upward. Similar stimulation of the upper side of the apical 10 mm. did not increase the geotropic curvature, but neither did it inhibit such bending. Later experiments (39), in which an electrical vibrator was used for mechanical stimulation, demonstrated that the magnitude of the transverse electrical polarity, its rate of change, and the rate of growth curvature are dependent on the duration of the mechanical stimulation. (The duration of stimulation is actually a measure of the number of weak taps that were applied.) These experiments indicate that mechanical and geotropic stimulation are additive. Similar results were obtained when only the apical 5 mm. were stimulated instead of 10, as in the previous experiments.

At this time it is worthwhile to note that the direction of the subsequent bending can be predicted from the orientation of the transverse electrical polarity, which is established by gravity, mechanical stimulation, or a combination of the two. Though it has not been conclusively proved, there is an indication that mechanical stimulation induces curvature by altering the transport of auxins. It seems that the zone of curvature migrates from the apex toward the base. This implies that the auxins are transported away from the stimulated and electronegative side of the plant. The Cholodny-Went theory of growth curvature includes the necessity of a transverse "polarization" of the cells to bring about the unequal distribution of the auxin (56). All of the above facts seem to be compatible with the Went-Kögl scheme of "electrophoretic" transport, but extreme caution should be exercised in making far reaching conclusions.

Mechanical plus geotropic stimulation of coleoptiles, from which the apical 3 mm. had been removed for 130 min., gave no growth curvature (39). Such stimulation, however, causes the seedling to establish a transverse electrical polarity, which is about the same as in intact plants. These results indicate that the presence of auxin is not required to establish an electrical polarity in the Avena coleoptile.

At least one attempt has been made to analyze the twining response by the use of the klinostat. Hendricks (21) found that a number of stems (morning glory, hop, and black bindweed) continued to twine even when they are rotated on a klinostat for 48 hr. so long as the angle of inclination was less than 75°. A further increase in the angle of inclination resulted in a gradual decrease in twining, until at 90° (horizontal) twining rarely occurred. Further experiments are necessary before these results can be clarified.

Electrotropism

Much of the older work on bioelectrical correlation in plants, as related to auxin and its transport, has been organized and presented by Thomas (54). Since the time of this publication a number of additional papers have appeared. Some of these will be considered here in an attempt to evaluate the present status of electrotropisms.

Since the tropic responses to electrical current are directly dependent on the effect of current on growth, work of this nature will be examined first. The experiments of Wilks & Lund (60) have confirmed earlier observations that elongation of the Avena coleoptile can be altered by longitudinally applied direct current. They report that current of the order of one microampere reversibly inhibits elongation when the flow is from the apex toward the base. The degree of inhibition is dependent on the strength and duration of the current. [The interested reader will find related and similar results in (16, 17, 26)]. Deviations from normal growth of onion roots, when current of low intensity was passed continuously through them, were observed by Berry, Gardiner & Gilmartin (3). With an IR drop of about 15 mv. per mm., lateral roots were produced quicker and in greater numbers than in controls. Histological sections revealed that these lateral roots originated in the pericycle, and that they resulted from normal mitosis. An IR drop of 28 mv. per mm. caused abnormalities in roots such as local swellings, doubling of individual roots, looping, spiraling, disorganization of cells of the central cylinder, enlargement of cortical cells, and extreme vacuolization. These last mentioned abnormalities were not dependent on the polarity of the applied current.

Studies of a number of growth responses, involving orientation, to applied electrical current have recently appeared in the literature. Germinating pollen tubes of *Vinca rosea* emerge and grow predominantly toward the cathode when placed in an electrical field (27). The percentage of tubes emerging toward the cathode reaches the maximum at a current density of about 550 microamperes per sq. mm. Higher current densities result in reversible inhibition of growth, which depends on the polarity of the current.

Inhibition toward the cathode begins at about 550 microamperes per sq. mm., while inhibition toward the anode requires a current density of 800 microamperes per sq. mm. Onion roots have been observed to orient toward the anode of a transversely applied electrical field of 0.8 to 3.5 microamperes per sq. mm. by Marsh & Beams (28). This curvature, which is reversible, varies with the current and duration of application.

Direct current of 5 to 20 microamperes applied transversely for 2 min. to the 5 mm. apical segment of the Avena coleoptile induces bending toward the positive pole of the current-applying circuit. The rate and magnitude of such curvature are dependent on the intensity of the applied current (42). The experimental evidence indicates that the observed bending is due to growth processes. Later Schrank (43) demonstrated that 10 microamperes of direct current would induce curvature when applied for 10 min. to 2 mm. segments at any level below the apex. As the distance between the apex and the current-applying contacts is increased, the magnitude of curvature induced by a given current decreases, and the time required to reach the maximum curvature in the initial direction increases. These facts extend support to the idea that the current produces its effects via the auxin mechanism. The data clearly show that the zone of curvature moves toward the base, thus indicating that the auxin is transported across the coleoptile by the applied current. Furthermore, these experiments (43) show that the initial bending, in and above the contact region, is toward the positive pole of the current-applying circuit. This is followed by a subsequent bending, which is basal to the contacts, and toward the negative pole.

Inherently the Avena coleoptile has no transverse electrical polarity (41). Application of current always establishes an electrical polarity, which apparently is not dependent on the presence or distribution of the auxins (43). It is noted that the initial curvature due to current is always toward the side that was made electropositive by electrical stimulation. In previously discussed tropisms, the curvature was always toward the electronegative side of the plant. So far this investigator has not published data or an hypothesis to explain these observations.

Data demonstrating for the first time the important relationship between phototropism and curvature induced by transversely applied current were published by Schrank (44). These experiments show that 10 microamperes of direct current applied for 2 min. at a level 5 mm. basal to the apex induces an initial bending away from 200 meter-candle-seconds of light when the positive contact of the current-applying circuit is placed on the shaded side of the plant. When the unilateral illumination is reduced below maximum effectiveness (100 meter-candle-seconds), 10 microamperes of current applied for one minute with the positive contact on the lighted side increases the magnitude of bending toward the light. These results were interpreted to indicate that electrotropic and phototropic bending are restricted by a common limiting factor, that the stimuli are to some extent additive, and that current and illumination apparently influence the same curvature mechanism.

It has been known for some time that curvature can be induced in the coleoptile by applying current longitudinally to one side (15). This observation has recently been confirmed and extended (45). Originally it was thought that this electrically-induced curvature was independent of the polarity of the applied current (15), but more recently it has been consistently demonstrated that the magnitude of curvature, as well as the temporal sequence of events, is definitely dependent on the polarity and strength of the longitudinally applied current (45).

In this connection it becomes worthwhile to note that direct current (10 microamperes for 2 min.) applied longitudinally to the apical 5 mm. of the Avena coleoptile always inhibits bending toward 200 meter-candle-seconds of unilateral light (46). The preliminary experiments of Wilks (60) have also demonstrated that longitudinally applied direct current alters phototropic bending. As before, the magnitude of curvature inhibition and the temporal sequence of events are dependent on the polarity of the applied current, the type of contacts, and the position of the contacts with respect to the source of illumination.

While admitting that not all of the steps in the sequence of events have been fully proved, Showacre & duBuy (47), in order to include curvature responses to electrical stimulation, have expanded their scheme of tropisms. The energy of stimulation, regardless of the type, causes retardation of protoplasmic streaming on the side of the plant oriented toward the source of the stimulation. This, in turn, causes a greater portion of the auxin produced in the apical cells to move to the opposite side and induce bending. There are data that are consistent with this concept (47), but on the other hand, there are additional facts that do not fit into this hypothesis.

The Showacre-duBuy concept is inadequate to account for several observed facts, including the following: (a) Clark (13) has reported that protoplasmic streaming can be inhibited by saponine (0.05 saturated) without affecting auxin transport, but, conversely, sodium-glycocholate (1:5,000) completely stops auxin transport without changing protoplasmic streaming; (b) electrotropic bending [current applied longitudinally (45) and current applied transversely (42, 43)] is certainly dependent on the polarity of the applied current while protoplasmic streaming apparently is not (15); and (c) current applied longitudinally either to the illuminated or to the shaded side of the oat seedling always inhibits phototropic curvature, and this inhibition is also dependent on the polarity of the current (46).

Perhaps the fundamental reason for the expenditure of space and effort on current-applying experiments should be clarified. It is a matter of common knowledge that living polar systems are surrounded and permeated by continuously maintained electrical fields which are generated by the constituent cells. The frequently suggested function of these complex and intricate fields is that they serve as the directive forces which are essential in oriented cellular processes. A logical and direct experimental approach to the function of these electrical fields is to alter them by superimposing fields of external origin and known orientation. When this is done with the Avena coleoptile

as the experimental material, the results which have just been reviewed support the conclusion that the experimentally applied electrical field brings about its effect on growth by affecting the auxin mechanism. The auxin controlled process then remains the common intermediate step in all of the tropisms. Experiments of this nature further indicate that the imposed field apparently influences the transport of auxin, but that the mechanism seems to involve more than simple electrophoresis.

RÉSUMÉ

In its broad and inclusive aspect the tropism phenomenon can be considered to include the following three processes: (a) the synthesis of auxin; (b) a mechanism to control or account for the unequal distribution of the auxin throughout the organ concerned; and (c) the precise relationship of auxin to growth. As was indicated in the introduction, the primary concern has been with the second of the above features. More and more evidence has accumulated, as indicated by the experiments that have just been reviewed, to show that an unequal distribution of auxin is a required step in all of the tropisms. The chief problem that remains in this connection is to account for this unequal distribution. In phototropism, additional study has been given to the role of photo-destruction of auxin, and some attention has been directed to the fact that illumination in some instances alters the rate of auxin synthesis. However, lateral transport of auxin with little information to identify the directing mechanism, still seems to occupy the favored role. In the other tropisms, the transport of auxin remains about the only suggested method to account for its unequal distribution. A correlation force to explain this transport has not been definitely established, but a number of experiments were cited which tend to indicate that the inherent electrical field could be the fundamental mechanism to accept the role of the required oriented forces for the Cholodny-Went theory of tropisms.

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ORGANIC ACID METABOLISM

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Introduction

One of the principal difficulties in reviewing the metabolism of organic acids in plants is to determine the boundaries of the subject. The organic acids lie at a crossroads in plant metabolism. As Chibnall (1) puts it, "it is the group of substances we refer to as organic acids . . . that occupy the central and therefore the key position, in the carbohydrate, protein and fat metabolism of plant cells." In addition to functioning in catabolic changes, the organic acids produced by the dark fixation of carbon dioxide apparently act as important intermediates in photosynthesis. Organic acids may be accumulated in quantity as end products of metabolism; furthermore, the oxidation of organic acids has a close connection with growth. For the present review attention has been concentrated on the organic acids themselves, and their occurrence, and rôle in catabolism and growth. Interrelationships with the metabolism of other compounds have been principally studied with microorganisms and animal tissues, while the function of organic acids in photosynthesis is discussed in another review.

The literature to 1940 has been covered by two previous reviews (2, 3) and there is also a recent brief discussion by McKee (4). The work with other than plant tissues has been reviewed recently and thoroughly (5, 6).

Determination and Separation

General.—The methods employed for the extraction and determination of various organic acids are essentially those discussed in previous reviews (2, 3); however, some valuable modifications have been introduced. Pucher *et al.* (7) have revised and summarized their methods, including a microprocedure for the extraction and determination of total acidity, oxalic, malic, and citric acids. Broyer *et al.* (8) have discussed the effects of pretreatment on the total acidity of barley roots and recommend the use of freshly expressed sap for such determinations. They have used a liquid extraction method for the preparation of the organic acid fraction from such expressed saps (9); this method is also applicable to plant tissues in general, although it suffers from a very long extraction period (56 hr.). In the authors' laboratory it has been found that drying causes a considerable increase in the keto acid fraction of seedling material. In their experience the most reliable methods of preparing plant tissues for analysis are either: (*a*) by grinding the fresh, thoroughly chilled tissue, incorporating this material with asbestos [as was proposed by Pucher *et al.* (7) for dried tissue] or, (*b*) by lyophilizing the tissue and then grinding it. Extraction is with a Soxhlet extractor in either case, and need be run only overnight. The A.O.A.C. (Association of Official Agricultural Chemists) methods for the determination of total

acidity in fruits and fruit products have been investigated and reviewed by Bollinger (10, 11).

Isherwood (12) has devised an extraction procedure based upon the chromatographic washing of the acidified crude extract in a silica gel column and this method has been used successfully by a number of workers.

Individual Acids.—Various chemical methods for the determination of malic acid have been proposed and reviewed (7, 13 to 17). Suomalainen & Arhimo (18) have shown that the 2,4-dinitrophenyl hydrazone obtained from the oxidation of malic acid according to the method of Pucher *et al.* (7), is the 2,4-dinitrophenyl hydrazone of glyoxal and that it arises from the oxidation of malic and aspartic acid, β-alanine and tyrosine. This method, in the authors' experience, has proved to be the most sensitive of all the malic acid determinations. A manometric method in conjunction with the determination of fumaric acid has been proposed by Krebs *et al.* (19). Another manometric method based on the bacterial oxidation of malic acid to lactic acid and carbon dioxide has been proposed by Rentschler (20) and applied by Gachot (21). The development of this method would appear to be most rewarding.

The micro-determination of citric acid has been considerably discussed (7, 15, 22 to 25) and is best described by Umbreit *et al.* (26). A thorough study of the colorimetric and titrimetric methods of Pucher was made by Breusch & Tulus (27), who found that the former method was the better and was the more specific for citric acid.

New methods for the determination of succinic acid have appeared (13, 14, 17, 28), but it seems clear that the succinoxidase method [described by Umbreit *et al.* (26)] is the best for this acid. Similarly, methods for the determination of isocitric acid and aconitic acid have appeared (15, 29, 30). Probably the simplest of these methods depends on the conversion of isocitric and aconitic acids into citric acid with aconitase; the respective concentrations can then be calculated from the equilibrium constants involved. The exact values of these constants, however, have been subject to some discussion (29, 32, 33, 34). According to Johnson (33), the equilibrium mixture contains 80 per cent citric acid, but Krebs & Eggleston find 89.5 per cent and Vickery & Abrahams (34) recently also found 89 per cent.

Two modifications for the detection of tartaric acid have appeared (13, 14). Krebs *et al.* (19) have proposed a manometric method for fumaric acid. The determination of oxaloacetate has been reviewed by Virtanen *et al.* (35, 36), who show that if alkali is added to the leaves before macerating, decomposition is prevented. The determination depends on decarboxylation with aniline. Krebs (37) has shown that the decarboxylation goes spontaneously with an optimum at about pH 4 and is greatly accelerated by aluminum, copper, ferric, and ferrous ions. Villano & Langella (38) have described a method for α-keto glutaric acid. The micromethod of Lu (39) for pyruvic acid has been modified (40, 41) and now appears to be very reliable. New methods and modifications of old ones are presented for oxalic acid (7, 13), lactic acid (42, 43), and volatile acids (14, 16, 42). Many of the above men-

tioned methods are described by Umbreit et al. (26). Acetic acid seems to have been somewhat neglected, as the published methods are usually unsuitable for it in presence of other volatile acids, unless the quantities available are sufficient for a Duclaux distillation. However, the method of Caselli & Ciaranfi (44) seems promising and should be specific, though rather complex.

As has been pointed out by many authors, the actual proof of the occurrence of a specific acid in plant tissues is often not given by its determination, but only by its actual isolation and identification. Methods for the qualitative recognition of various organic acids are given by Denigés (45). The separation of various acids by salt precipitation and by the classical ester distillation method of Franzen are still widely and successfully used. A modification has been made in the latter method (unpublished), which involves esterification with diazomethane (or diazo-ethane). By this procedure one obtains the methyl (or ethyl) esters at room temperature, thus omitting the harsh treatment with hydrochloric acid at elevated temperatures.

A method for the separation of organic acids by partition chromatography has been proposed by Isherwood (12) and has been successfully used in a modified form by Burris & Stutz (private communication). Benson et al. (46) have separated five carboxylic acids by two-dimensional paper chromatography. The method is particularly applicable to radioactive tracer work. A more general chromatographic method, in which the ionization of the acids is suppressed by an excess of formic acid, has been described by Lugg & Overell (47). The R_F values for 14 organic acids are given, and an experiment with carrot extracts shows the practicability of the method. In a recent communication dealing with the separation and identification of organic phosphate esters by paper strip chromatography, Hanes & Isherwood (48) mention also that in preliminary experiments they have been successful in separating various organic acids by essentially the same techniques. When developed, these methods should provide a very valuable tool for micro-separation and identification of organic acids.

THE ORGANIC ACIDS OF LEAVES

Succulents.—The large diurnal change in acid content of various crassulacean plants, a phenomenon known as crassulacean metabolism and associated with the succulent habit, has received considerable attention during the last few years. The basic observation, which has been repeatedly confirmed, is that the acid content of these leaves increases during the night and decreases during the day. [For literature prior to 1940, see (3)]. One of the principal acids of various succulent plants, which in the past has been known as "crassulacean malic acid," was independently identified as isocitric acid by Nordal (49) and by Pucher and Pucher & Vickery (50, 51), and this was confirmed by Krebs & Eggleston (29).

A series of studies on the metabolism of excised *Bryophyllum calycinum* leaves has been made by Pucher et al. (52 to 55). This very thorough work suffers from the fact that, until the last group of experiments, only limited

attention was devoted to temperature control, and much of the work was carried out at a temperature higher than that optimal for acid formation in *Bryophyllum* species. Total organic acidity and malic acid were found to accumulate linearly in the leaves and stems of *B. calycinum* during a growth period of 95 days; accumulation of citric and isocitric acids slowed off toward the end of the period. Apparently isocitric acid, which represented about 18 per cent of the total organic solids, accumulated as rapidly in *Bryophyllum* as did malic acid in tobacco (in the authors' previous studies), while citric acid accumulated at approximately the same rate in both plants. Oxalic acid, which is a major acid component of tobacco, is essentially absent in *Bryophyllum*. This appears to be the case in several other succulent species (30).

In a study of the relation between starch and organic acids in *Bryophyllum* leaves, Pucher *et al.* (53, 54) carried out experiments of two kinds, one with leaves harvested from plants which had been grown during the winter in a greenhouse, and the other with leaves cultured in water in the dark or light or in alternate light and dark periods. The former leaves were thus grown at a more or less high and constant night and day temperature, while the latter when cultured in the light were at 20 to 27°C., and when in the dark at 21 to 23°C. These temperatures are very high, as was noted above, for it has been shown by Wolf (56) that acids are used up in *Bryophyllum* leaves kept in the dark for long periods at 20°C. or higher. However, it is true that Thoday & Richards (57) found that when *Kleinia* was kept in the dark at 25°C., the malic acid remained at a high level. In the work of Pucher *et al.* (54) the malic and citric acids showed the classical diurnal variation with leaves cultured in alternate light and darkness, but isocitric acid constituted an exception, since it decreased at night and increased during the day. On the other hand, Krebs & Eggleston (29) and Bonner & Bonner (30) found small increases in isocitric acid in the dark in *Bryophyllum* species.

Pucher *et al.* (53, 54) confirmed and extended the finding that starch varied in a manner converse to that of the organic acids, i.e., during the period of acid formation in the dark starch was used up, and during the period of acid disappearance in the light, starch reappeared. Whether starch is directly formed from organic acids (rather than by photosynthesis) is, however, far from certain. The only indication that this might occur is their observation that leaves collected in the morning, i.e. at their peak organic acid content, and cultured in complete darkness, showed a loss of about one half of their total acidity in 48 hr., during which time there was a small but pronounced formation of starch (6.4 gm. per kg. fresh wt.). It is to be expected, of course, that acids would be used up in the dark at 20 to 25°C., but if their conversion to starch is real, this would be powerful evidence that the reactions relating carbohydrates to organic acids are indeed fully reversible under natural conditions [cf. the postulated conversion of malic acid to carbohydrate in the beet (131)]. However, these workers did not observe a formation of starch in the dark quantitatively comparable with that observed in the light. They state that owing to the insignificant increase in

total organic solids during the day, photosynthesis can play only a small rôle in the formation of starch. However, it seems that the increase in weight due to photosynthetic formation of carbohydrates may be largely offset by the loss of weight to be ascribed to organic acid disappearance.

A series of experiments in which temperature was controlled during the dark culture of excised *Bryophyllum* leaves is presented in the final paper of this series (55). Here they confirmed previous observations [(30) and literature cited therein] that the optimal temperature for acid formation is far below 20°C. and closer to 10°C. Decreasing the culture temperature from 20°C. to 9°C. resulted in a great increase of acid synthesis and at the same time an increase in the quantity of starch consumed. At 20° C., during a 24-hour period, starch decreased 8.4 gm., while 11 gm. of organic acid were formed. At 9°C. these figures were 11.1 gm. starch decreased to 12 gm organic acid formed, and at 1°C., 6 gm. starch decreased and 16 gm. organic acid formed (66 hr.). Hence, there is no simple relation between starch and organic acids, since at the low temperature there is insufficient starch consumed to account for the acids formed. Thus the authors state: "starch may be the source of much of the acid, in certain cases of all, but it is not the only possible source." There are many more interesting features of this paper but space does not permit their discussion.

That the organic acids of various succulent plants, at the optimal low temperature for acid formation, and in the dark, do not arise in the main from the oxidation of carbohydrates but by the actual fixation of carbon dioxide, was demonstrated by Bonner & Bonner [(30) *cf.* also (58)]. In their experiments there was shown to be an almost linear relationship, with low carbon dioxide concentrations from 0 to 0.1 per cent in air, between acid formation and the carbon dioxide partial pressure. At higher partial pressures, up to 10 per cent carbon dioxide in air, there were further small increases in organic acid content. The major part of the acid increase due to carbon dioxide fixation was found in malic acid. They were unable to abolish acid production in carbon dioxide-free air and it may well be that at the temperature used (3°C.) this residual acid formation represents that formed directly from the oxidation of carbohydrate (or polysaccharides). It seems indicated that at low temperatures acids are formed primarily by carbon dioxide fixation, whereas at the higher temperatures their formation may be predominantly oxidative. However, Wolf (56) has shown that when leaves are placed at high temperatures in the light in high concentration of carbon dioxide (50 per cent CO_2 in N_2) the expected disappearance of organic acid is greatly delayed. Since the dry regions in which succulents commonly flourish tend to have a marked fall in night temperature, it is likely that in nature their organic acids are formed predominantly by carbon dioxide fixation.

The fixation of carbon dioxide with the formation of organic acid by leaves of *Bryophyllum* in the dark was confirmed by Thurlow & Bonner (59) using carbon dioxide containing C^{14}. These authors showed that of the $C^{14}O_2$ originally given excised leaves of *B. crenatum*, 50 per cent of the activity was recovered in the organic acid fraction, which in this case is composed almost

wholly of malic, citric, and isocitric acids [*cf*. (30)]. Burris & Stutz (60) have obtained similar results with *B. calycinum* for which they have detailed analyses. In one experiment they found that after a 15 min. dark fixation period 90 per cent of the radioactive carbon was in the malic acid fraction. Incidentally, it is of interest that illumination did not change the acid distribution greatly, for after 15 min. photoperiod the malic still constituted 89 per cent of the radioactive fraction.

Sideris *et al*. (61) have shown a diurnal change of organic acid content in pineapple leaves. As in the case of *Bryophyllum* the major part of the change was in malate (75 per cent) accompanied by a similar but smaller change in citrate (37 per cent).

Harder's observations (62) on the morphology of *Bryophyllum* in different photoperiods are of interest here. Briefly, he found that plants kept in long days lose their succulent habit, the leaves becoming thin and larger in area. The succulence could not be restored by high salinity in the soil. This seems very suggestive in light of the experiments described above:—it may be that long-day conditions cause the disappearance of organic acids and that the loss of succulence is a result of this change.

Pucher *et al*. (52, 53, 63) in their studies of leaves have also followed the behavior of organic acids in the stem tissue. It is remarkable that in this tissue there was, in contrast to the leaves, no marked diurnal variation of acid content, but there was a pH change similar to that in the leaves. That there are some succulents, such as *Mesembryanthemum*, that do not behave in the usual manner has already been observed (30); but the surprising fact that the organic acids of the stem do not follow the usual pattern indicates that the bulk of the organic acid metabolism takes place wholly within the leaf and that there is very little transport outside the leaf. In this connection it is interesting that in the experiments with various amounts of nitrate and ammonium nitrogen (see below), the stem tissue responded exactly as did the leaf tissue.

Nonsucculents.—There have been several investigations dealing with the effect of nutrition on the organic acids of leaves. Vickery *et al*. (65) and Pucher *et al*. (63, 64) have studied the effect on the composition of tobacco. *Narcissus poeticus*, and *B. calycinum* of the form in which nitrogen is supplied. Growing whole plants, or the leaves and stems, on culture solutions which contained the same amount of nitrogen (ranging from 100 per cent nitrate nitrogen to 100 per cent ammonia nitrogen with several intermediate stages) they showed that in these plants total organic acids decreased with increasing ammonium nitrogen. The drop in some individual acids was much greater in *Bryophyllum* than in tobacco. They suggest that the lowest malate level found in the 100 per cent ammonium nitrogen plants represents the amount required for respiration. The data on *Narcissus* are particularly interesting in that this is the first recorded investigation of the organic acids for plants in this botanical group. Both the bulbs and leaves of developed plants were low in organic acidity, the highest values found being respectively 3.1 per cent and 0.4 per cent of the total organic solids. In contrast to tobacco,

Bryophyllum and rhubarb, where some or all of the readily determined acids (oxalic, citric, isocitric, malic, and succinic) are the major acids, *Narcissus* bulbs and leaves contained oxalic, malic, citric, and succinic acids in an amount that accounted for only half of the total organic acidity. The authors did not test for the presence of isocitric acid in these tissues and it is possible that here, as in *Bryophyllum*, this compound may be the bulk component. With *Narcissus* there was a marked enrichment of the tissues in malic acid following culture on nitrate nitrogen. Similar experiments to those just mentioned have been carried out by Vladimirov (66) and by Evans & Weeks (67) on tobacco; essentially the same results were obtained, namely that plants grown on ammonium nitrogen contained much less organic acidity than did those grown on nitrate nitrogen. Blackman & Templeman (68) also found that calcium nitrate increased the total organic acids in both grasses and clover. Pepkowitz, Gilbert & Shive (69) found that with oats grown with and without nitrate nitrogen total organic acids increased in presence of nitrate; the increase was partly malate and partly unknown acids, the latter being the largest fraction of the total organic acids. Oxalic acid formation was also dependent on the presence of nitrate, but the carbonyl acids showed higher values in plants grown in the absence of nitrate. These workers also investigated the effect of oxygen tension and found that the yield of total organic acids was higher at low than at high oxygen levels. There was no effect on malate formation, however, and the formation of oxalic acid was found to vary inversely with the oxygen tension. The fact that malate formation was not dependent on oxygen tension would seem to need further substantiation.

The influence of cations appears somewhat complex. Vladimirov (70) has reported that in tobacco and kok-saghyz the effect of potassium ions on the metabolism of organic acids depends on whether the plants were grown on ammonium nitrogen or on nitrate nitrogen. The presence of potassium causes the organic acid content of plants grown on nitrate nitrogen to fall, whereas in those grown on ammonium nitrogen, potassium causes an increase. This investigator also found that the presence of sulfate lowered the amount of organic acid, while chloride raised it. He concluded that ammonium salts plus potassium and magnesium sulfates should increase the content of reduced substances within a plant, while nitrate plus potassium or sodium chloride should increase the organic acid content.

Kurchatov (71) has reported that the citrate content of various plants was increased by potassium or phosphate, whereas Ward & Petrie (72) found added phosphate to depress the amount of total organic acids in tobacco. Iljin (73) has studied the relation between organic acids and calcium chlorosis in leaves of apple and cherry. Iljin (74) and Cooil (75) have investigated the relation between inorganic salts and organic acids in growing and in mature leaves. Total organic acids and citric acid were correlated with the amount of potassium in expanding leaves, but malic acid was more closely related to calcium; for example, in expanding and mature leaves of guayule (75), the highest value for malate was found when the maximum amount of calcium

was found [*cf.* the observation of Blackman and Templeman (68) above]. Iljin's extensive analyses on 10 species show that the total acids, malic and citric, increase both with age and with the advancing season. In general, the acids are correlated with the calcium content, especially for plants growing on a chalky soil. Autumn yellowing causes no appreciable reduction. Pierce & Appleman (76) have made a similar study on 12 common agricultural species, but found little correlation between various ions and the organic acid content. The most interesting feature of their work, which included analyses for total organic acids, oxalic, malic, citric, and unknown acids, was the high amount of unknown organic acids in these 12 plant species—in most cases amounting to 50 per cent of the total, or more.

In breeding experiments with tobacco (*N. tabacum, N. rustica*, and *N. glauca*) Kostov & Nikolov (77) found that tetraploid forms tended to have more citric acid than did the diploid. Since growing conditions are not mentioned it is of interest to know how much these differences were due to variations in light, temperature, and fertilizers.

Pucher & Vickery (78) studied the changes in organic acid composition in excised tobacco leaves cultured in water and in inorganic and organic salt solutions. Culture in water and in inorganic salts led to the formation of citrate with a corresponding decrease in malate, while the total acidity remained essentially constant. Culture in malate greatly stimulated the formation of citrate, while culture on citrate, succinate, or fumarate brought about increases in both citrate and malate. Culture on tartrate caused an increase in the unknown acid fraction, an indication of the accumulation of this acid. Leaves cultured on malonate showed an increase in total acids, (which in some cases amounted to more than could be accounted for by the uptake of malonate alone) an increase in citric acid and a still greater decrease in malic acid. Equimolar mixtures of fumarate and malonate caused a large increase in total acids, more than in any of the other tests, and citric increased while malic decreased. These experiments indicate that malonate (as would be expected) blocks the formation of malate. When this happens additional citrate would appear to be formed from the succinate which "backs up." Under normal conditions citrate is apparently formed from malate. Pucher & Vickery conclude from this work that those acids which are part of the tricarboxylic acid cycle enter readily into the metabolism of the tobacco leaf, and hence that enzyme systems capable of metabolizing these acids are present.

Some of the above findings were confirmed in the same laboratory with another variety of tobacco (34) and, in addition, leaves were cultured on D-isocitrate and on acetate. Isocitrate brought about an increased citrate content and at the same time a small decrease in malate. It is assumed that isocitrate is converted by the aconitase of the leaf to citrate, although if this were indeed the case, results similar to those obtained from culture on citrate should have been obtained, i.e. both citrate and malate should have increased. The presence of aconitase in tobacco leaves has not been demon-

strated. The leaves cultured on acetate became flaccid by the end of the culture period, in contrast to all other organic acids tested, but all of the acetate taken up from the culture solution had disappeared from the leaf tissue. There were no marked changes in total acids and it is presumed that acetate is completely oxidized, a conclusion also indicated by Krotkov & Barker's experiments (79) with radioactive acetate (see *Metabolism*). These results led Vickery & Abrahams (34) to conclude that culture on organic acids brings about a greatly increased respiration. Such a deduction is in agreement with direct measurements which are discussed below. In regard to the interrelationships between various organic acids, the experiments show that during culture in water, about two moles of malate are converted to one mole of citrate, which relationship also holds true during culture on malate. During culture on citrate, one to two moles of this acid form one mole of malate. Thus, when the system contains more than the usual amount of citrate the normal course of reactions which convert malate to citrate are considered to be reversed. Pyatnitskii (80, 81), in experiments where tobacco leaves were cultured in solutions of organic acids, cured, and then tested for organic acids, also concluded that citrate is formed at the expense of malate, but that the presence of magnesium ions is required for this to take place. These observations are supported by similar experiments of Grebinskii (82) in that during curing the increased citrate content was proportional to the loss of malate. In addition, the formation of citrate was further stimulated by the presence of a phosphate buffer (pH 7.8). This author also showed that girdling the stems caused a considerable decrease in these two acids, especially malate.

Wood *et al.* (83, 84, 85) and Cruickshank & Wood (86) have followed the organic acid content of Sudan grass, Kikuyu grass, and oats through a series of investigations concerning the metabolism of these leaves during starvation The behavior of the various metabolites was essentially the same in the leaves of all these in air. However, the behavior of malic and citric acids differed markedly from that described by Vickery *et al.* (87, 88) in earlier investigations with rhubarb and tobacco. In the grasses, starvation in nitrogen produced little or no increase in the malate and citrate content. In air, however, malate accumulated up to a late stage of starvation and then decreased, while citrate also increased to a maximum, which came earlier than that of malate, and then decreased. This was accompanied by an overall increase in the total acids. In tobacco, on the other hand, during starvation in air the total acids remained essentially the same, while malate decreased considerably and a corresponding amount of citrate was formed (78). In rhubarb there was a decrease in total acidity as well as in malate and citrate. Wood *et al.* feel that their experiments show that in grasses, organic acids and particularly malate, are not derived in appreciable amount from nitrogenous sources such as aspartic acid, since otherwise the proteolysis which accompanies starvation should have produced malate even in nitrogen. They propose, however, that the interconversion of organic acids in these plants

takes place via a tricarboxylic acid cycle which is closely connected to nitrogen metabolism via amides and amino acids, as has been proposed by various workers in the past, and is discussed in another section. They also suggest that the conflicting data between grasses, tobacco, and rhubarb may be reconciled by assuming that a tricarboxylic acid cycle is in operation in all cases and that the differences result from variations in individual enzyme activities.

It will be recalled that Warburg, as far back as 1886 (89) found a diurnal variation in acid content of various nonsucculent leaves. This observation has been confirmed and extended to a variety of leaves (90, 91), but aside from its mention there has been little basic investigation of this phenomenon. However, there is increasing evidence that carbon dioxide fixation, which may or may not be evidenced by diurnal variations in organic acid content, occurs widely in nonsucculent leaves. Recently, Burris & Stutz (60) have analyzed barley, tobacco, and tomato leaves which have been treated to a 15 min. dark fixation period in the presence of $C^{14}O_2$, following a period of preillumination. They found, for instance, that with barley 43 per cent and 47 per cent of the C^{14} was in the succinic and malic acid fractions respectively. In tobacco 88 per cent and in tomato 85 per cent of the C^{14} was found in the malic acid fraction. Succinic acid has not generally been included in leaf analyses, but an extensive survey (64) shows that it is widely distributed in plant tissues. However, in all cases it constitutes less than 1 per cent of the dry weight. Determinations made during the development of the tobacco plant indicated that even though this acid is present in only small amounts it may be regarded as one of the most active metabolites. This would agree with the above findings, and also with those of Calvin & Benson (see FRUITS) for dark reactions.

Vennesland and her collaborators (92 to 97) have made a valuable contribution to our knowledge of carbon dioxide fixation in their studies of β-carboxylases in higher plants. From parsley roots they have obtained and studied an oxaloacetic carboxylase, whose activities are associated with a malic dehydrogenase active with TPN; the system appears to catalyze two reactions independently, both of which have been shown to be reversible. These reactions are: (a) the reversible carboxylation of pyruvate with the formation of oxaloacetate, the well known Wood and Werkman reaction, and (b) the reversible reduction of oxaloacetate to malate, a reaction which requires as hydrogen donator Coenzyme II. This enzyme system is apparently very widespread, for Vennesland (96) has shown it to be present in 13 different species of higher plants, in roots, tubers, seeds, and leaves. Ceithaml & Vennesland (97) have made an enzyme preparation from parsley root which formed tricarboxylic acids in the presence of α-ketoglutaric acid and carbon dioxide. For this reaction manganese or cobalt ions are necessary. If Coenzyme II is added to this system, the initial fixation product is reduced to isocitric acid. All these data show the very widespread distribution of dark carbon dioxide fixing mechanisms in higher plants.

FRUITS

While there have been many investigations concerned with the isolation of organic acids from fruits (see section on OCCURRENCE AND DISTRIBUTION), detailed studies on the biochemistry of organic acid formation and metabolism in fruits are few. This is regrettable in view of the fact that many fruits apparently possess a very active organic acid metabolism. In addition, the amounts of organic acid present are frequently large, which should simplify the analytical aspects of such studies. The standard determination methods [reviewed by Bollinger (10, 11)], are generally adequate without micromodifications for such material.

Richards (98), from studies of the budding of various varieties on different rootstocks, has reported that the rootstock exerts a definite influence on the citric acid content of citrus fruits. There have been few studies of the influence of nutrition on organic acid content, like those reported above with tobacco. An increased citric acid content in grapefruit due to magnesium or potassium fertilization has been observed (99). Sinclair & Ramsey (100) have followed the organic acid content of developing Valencia oranges for an eight-month period. Their results indicate that the formation of organic acids is completed early in the development of this fruit and that there is little change during maturation. In the grapefruit the main change is decrease in free citric acid and increase in citrate. The studies of Sinclair and co-workers on other citrus fruits (170, 171, 173, 175) are summarized briefly in the Table under CITRIC ACID. Peynaud (101, 102) has made a considerable study of organic acid relationships during ripening of grapes, of which the major acids are tartaric, malic, and citric. Unfortunately there is little to indicate the rôle of tartaric acid, which he found to rise to a rather high concentration as the grapes ripen. It appears that malic acid decreases markedly during ripening, especially if the temperature is high (103). Flanzy (103) found the same thing true to a lesser extent for tartaric acid. Peynaud's work has been discussed by Causse (104) who disagrees with some of Peynaud's conclusions as to the origin of the three major acids. The latter author considers that during the ripening period, tartaric and malic acids arise from lactic acid while citric acid arises from acetic acid. In view of the rarity of lactic acid in plants, this hypothesis, and perhaps the views of both these authors, will require further experimental verification.

In apples, Tavernier & Jacquin (105) found that malic acid, which is 90 to 95 per cent of the total acids, falls during ripening to about a third of the value in the early June fruit. The citric and succinic acids, 3.6 and 0.6 m.eq. respectively per liter of juice, showed no decrease at all during the whole season, which means of course that the total amount increased in proportion to the volume of tissue. In pears, the same authors (106) found surprisingly large amounts of citric acid. In table varieties, it appears, citric acid accounts for only 5 per cent of the total acids (the rest being malic), but some varieties grown for pressing have more citric than malic. It is pointed out that the juice of these would contain 8 gm. citric acid per liter, while the law (no doubt intended to cover adulteration) allows only 0.5 gm.

GENERAL BIOCHEMISTRY OF ORGANIC ACIDS IN PLANTS

As was mentioned in the Introduction, it is now impossible to consider organic acids in plant tissues without taking into account almost every aspect of respiration and metabolism. Many workers have stressed the importance of organic acids in nitrogen metabolism (1, 107), and there have been two recent and excellent reviews on this subject (1, 4). Braunstein (6) presents a diagram of various metabolic processes, which stresses the rôle of organic acids not only in the formation, interconversion, and breakdown of amino acids, but also their central position in respiratory hydrogen transport, aerobic phosphorylations, the fixation and liberation of carbon dioxide and ammonia, and the synthesis of specific nitrogenous cell substances. However, actual observations on all such interrelationships in higher plant tissues are scarce.

Transamination and amide formation.—An important link between organic acids and nitrogen metabolism is, of course, transamination, but the evidence for such reactions in higher plants has not been greatly expanded since 1940, and is very incomplete. Cedrangolo & Carandante (108) found that extracts of graminaceous plants were much more active than similar extracts of leguminous plants in promoting transaminations between aspartate and pyruvate, aspartate and α-ketoglutarate, and alanine and α-ketoglutarate. Virtanen & Laine (109) have investigated transaminations involving glutamine and asparagine. Using crushed pea plants, which transaminated pyruvate to alanine in the presence of glutamate or aspartate, they found that when the amides were substituted for the latter amino acids, the final amount of transamination carried out was greatly depressed. Measurements on the amide nitrogen split off during the period of transamination indicated that, in the presence of amides, transamination proceeds through the corresponding dicarboxylic acids. Rautanen (110), in a very short report, states that many green plants (the material used is not mentioned) are capable of carrying out transaminations of aliphatic, but not of aromatic, amino acids. Such reactions are stated to reach a maximum in 40 to 60 min., the optimal conditions being 41°C. at pH 6.9. A survey by Leonard & Burris (111) of the glutamate-aspartate transamination showed this reaction to be carried out by a variety of higher plants, in leaf, stem, root, fruit, and nodular tissue. These authors report that the transamination rate, per unit of tissue, decreases with increasing age in a growing plant. Albaum & Cohen (112) have followed transamination activity in developing oat seedlings. In this object the glutamic aminophorase activity rises rapidly from the second day following germination. Rautanen (113) has investigated the formation of amino acids and amides in *Pisum sativum* and has again demonstrated the close relationship between the formation of these substances and the organic acids, in this case especially malic acid. One of the main problems studied by Wood *et al.* in the experiments already discussed (83, 84, 85) was to what extent the amides arise directly from proteolysis—an old problem [*cf.* (1)]. The results were clear, since starving leaves in a

nitrogen atmosphere produced amino acids but very little amide, and almost no organic acid. In air there was a large rise in asparagine, which was preceded by a rise in malate and citrate.

Metabolism of individual acids.—There have been some very interesting observations on the biological oxidation of oxalic acid. This acid has long been considered a by-product of plant metabolism, which when once formed was not readily used up. Hence the finding of Franke *et al.* (114) that the moss *Hylocomium triquetrum* and the leaves of several higher plants contain an oxalodehydrogenase is remarkable. These plants include sorrel, rhubarb (a plant known to store large quantities of oxalic acid), clover, and spinach. Purified enzyme preparations were prepared and studied. The enzyme from the moss differs from that in higher plants in its thermostability and its temperature and pH requirements, but all the preparations have remarkable stability toward heat. The enzyme is inhibited by sodium nitrate (6.7 $\times 10^{-5}M$), but not by cyanide, sulfide, or azide. A different type of oxalate oxidation has been demonstrated by Blom & Niekerk (115) who showed that suspensions of mosses brought about the oxidation of oxalic acid to hydrogen peroxide and carbon dioxide. This reaction is inhibited by cyanide or by lack of oxygen. However, as this was in no way a purified system, the inhibition by cyanide is no proof that this enzyme differs from that of Franke *et al.* It is to be hoped that enlarging the scope of such work will clarify the obscure position of oxalic acid in plant tissues.

The utilization of acetate by tobacco leaves has been studied in a preliminary manner with carboxyl-labeled acetate by Krotkov & Barker (79). In a short-term experiment a portion of the labeled carbon appeared in a water-soluble non-volatile fraction, the properties of which suggest one of the dicarboxylic acids. In longer experiments, the labeled carbon was obtained as respired carbon dioxide. These authors feel that their experiments indicate the possibility of acetate utilization in higher plants, but do not prove it to be a normal metabolite. Evidence for the normal utilization of acetate in coleoptiles has been obtained in the authors' laboratory and will be discussed in the section on GROWTH.

Dihydroxy-maleic acid, whose presence in plant tissues has often been discussed, has been shown by Kuzin & Doman to be very rapidly oxidized to diketosuccinic acid when infiltrated into leaves of *Tradescantia* (116). This reaction is carried out only in whole leaf tissue or very freshly prepared leaf macerate to which dihydroxymaleic acid has been added. It is to be noted, in view of the apparent ease of the reaction, that in the above work the authors were isolating, in effect, the added dihydroxymaleic acid, and hence the disappearance of this substance when infiltrated into leaves might be by a reaction similar to that described by Ochoa (117), in which diketosuccinic acid is converted to α-ketoglutaric acid by carbon dioxide fixation. Dihydroxymaleic acid markedly increases oxygen uptake by crown-gall tissue (151).

Glycolic acid is formed when leaves are exposed to light in the absence of carbon dioxide (118). Oxygen is apparently necessary for its formation. It

disappears in the dark with great rapidity. Experiments on its rôle in respiration and the oxidation of chlorophyll are discussed in the next section.

Respiration.—During the past ten years a limited amount of work has been directed at uncovering evidence for the participation of a tricarboxylic acid cycle in plant respiration. The first fundamental step in this direction was taken by James and co-workers (119, 120) who demonstrated that pyruvic acid arose from hexoses through a phosphorylation cycle basically similar to the reactions of yeast. This was shown in barley roots and leaves and in cell-free sap; in each case, by using various aminonaphtholsulfonic acids, which have been shown (121) to be specific inhibitors of carboxylase, they were able to isolate pure pyruvic acid. Through studies on respiration and its inhibition, Bonner & Wildman (122) have deduced the conversion of hexose to pyruvate in spinach leaves, similar results being obtained with the Avena coleoptile (123). The presence of malic and isocitric acid dehydrogenases in the spinach leaf was demonstrated (122); these two acids, as well as succinic, fumaric, and pyruvic acids, can be used as a respiration substrate by starved leaves. In addition, fluoride inhibition of respiration was overcome by added pyruvate. Malonate inhibition was overcome by the addition of succinate, fumarate, malate, or isocitrate. All these facts taken together very strongly suggest the participation of a tricarboxylic acid cycle in respiration. In the case of the *Avena* coleoptile (123) the results were not as clear-cut. Various organic acids enhanced the oxygen uptake, in confirmation of the earlier finding of Commoner & Thimann (124), but fluoride inhibition of respiration was only partially overcome by various organic acids, although there was a slight accumulation of succinate during malonate inhibition. Here again there is evidence for an acid cycle, but its nature remains to be elucidated further. Berger & Avery (125) have shown the presence of several dehydrogenases, but not succinic dehydrogenase, in this tissue.

An approach of a slightly different sort has been made by Laties (126, 127). In barley roots the oxygen uptake was the same in glucose or pyruvate; respiration was inhibited by fluoride and this was relieved by pyruvate. Respiration was also inhibited by iodoacetate and under suitable conditions this too was relieved by pyruvate. With malonate inhibition, the respiration could be reestablished by a variety of organic acids. Under suitable conditions succinate accumulation (in both barley roots and spinach leaves) was found in the presence of malonate, but the amount of accumulation was dependent on the duration of the experiment and the concentration of malonate. With increasing time, or slightly increased amounts of malonate, succinate, after having first accumulated to some extent, disappeared. It is possible that malonate is metabolized in this tissue. The accumulation of succinate could be enhanced by pyruvate, fumarate, or both, under conditions which would indicate their participation in the oxidative formation of succinate. Such a demonstration of the oxidative formation of succinate in leaves and roots is a very strong argument for the involvement of a tricarboxylic acid cycle in higher plants.

Machlis (128) has also studied the respiration of barley roots in connec-

tion with salt uptake. In this work he was able to reverse iodoacetate inhibition of both respiration and salt uptake by organic acids, but these same substances had no effect on malonate inhibition, in contrast to the studies mentioned above. In addition to an increase of respiration in presence of organic acids there was also a greatly increased uptake of bromide in their presence. Salt uptake of wheat roots is inhibited by 2,4-D but with no significant change in respiration, yet citrate partially reverses the inhibition (129). Similar studies have been carried out on excised tomato roots (130), but in this case there was no inhibition produced by either malonate or fluoride, though iodoacetate caused an inhibition, which was not reversed by any of the substances tried. Organic acids did promote the oxygen consumption in tomato roots. Using beet root disks, Bennet-Clark & Bexon (131) found that the respiration slowly increased with time to a maximum. This increase was more rapid in potassium chloride or calcium chloride than in water, but was instantaneous (80 to 120 per cent) when expressed beet root juice was added. The factor in the juice causing this effect was shown to be malate and could be duplicated by citrate and other organic acids. Turner & Hanly (132) have shown that the effects of succinate on the oxygen uptake of carrot root slices are highly pH-sensitive. At an optimal succinate concentration, a maximum stimulation of oxygen uptake was found at a low pH; under such conditions a gross R.Q. of 2.0 was found. At higher pH values the stimulation of oxygen uptake was less, giving R.Q. values less than 1.

It is interesting that a survey of succinic dehydrogenase (133) showed this enzyme to be present in only a fraction of the plants investigated. It was also not found in Avena coleoptiles (134). The failure to find this enzyme in many cases may well be due to difficulties in its preparation, but the work emphasizes the need for caution in assuming the universal presence of organic acid cycles in plant respiration. Das & Sen Gupta (135) have made a survey of fumarase and succinic dehydrogenase in ungerminated and germinated seeds and have found these two enzymes in several plants. In germinating seeds, where there was a rapid increase of succinic dehydrogenase activity, they found relatively little effect of malonate, a concentration as high as 0.1 M causing only 29 per cent inhibition of respiration. Turner & Hanley (136), in a study of malonate inhibition in carrot roots, report a profound effect of pH on the inhibition, comparable to the effect on succinate oxidation mentioned above. At pH 4 there was good inhibition of respiration in the presence of 0.05 M malonate while at pH 7 in the same concentration of malonate they report an actual stimulation of oxygen uptake. These authors ascribe such effects to the degree of dissociation of the acid, or its salts, and hence to its rate of uptake. Experiments in the authors' laboratory show that malonate is indeed taken up by coleoptile tissue at pH 7, as shown by its ability to protect against iodoacetate inhibition. This effect of pH may be greater in roots than in other tissue, since Lundegårdh (137) finds that the effect of fluoride on salt absorption is also highly pH-sensitive, which is unexpected for so strong an acid.

Kolesnikov (138) has demonstrated a very large catalytic effect of gly-

colic acid on the oxygen uptake of ground barley leaf centrifugates, which is not given by other acids tried. At the same time it was shown that chlorophyll disappeared at a rate which was dependent on the concentration of glycolic acid present (57 per cent disappearance in $10^{-3}M$ glycolic acid). This author indicates that in addition to the glycolic acid oxidation system, there are three other systems involved in the oxidation of acids in leaves. Ulrich (139, 140) has shown that, as was expected, in excised barley roots the R.Q. was less than one during the formation of organic acids and greater than one during their disappearance. Their formation and disappearance was regulated by the cation-anion balance of the nutrient solution, i.e. when cations were absorbed in excess of anions, organic acids were formed, while organic acids disappeared when anions were absorbed in excess of cations. These experiments produced no evidence for the formation of organic acids by oxidative deamination of amino acids. It is worth noting that the formation of organic acids was reduced at 25° and almost prevented at 35°; considering the close parallel to the similar behavior of succulents it may be suggested that carbon dioxide fixation was a primary factor in acid formation (see section on *Succulents*).

Growth

A number of experiments indicate a relationship between organic acid metabolism and growth. While its exact nature is as yet far from clear such a relationship has been demonstrated at satisfactorily low physiological concentrations of the acids and evidently involves their oxidative metabolism.

In a study of the elongation of isolated Avena coleoptile sections in presence of indoleacetic acid and sucrose, Commoner & Thimann (124) found growth to be inhibited by iodoacetate. When potassium malate, succinate, fumarate, or pyruvate was added as well, the inhibition was completely prevented. It was also observed that the organic acid salts mentioned above definitely promoted growth in presence of auxin, and that pretreatment with them allowed auxin to cause subsequently an increase of oxidation rate. These observations have been many times confirmed and extended. Albaum & Commoner (141) and Albaum & Eichel (142) obtained similar results with whole Avena seedlings to which the solutions were applied via the roots, while Bonner (143) has recently confirmed the promotion of growth of coleoptile sections by malate, fumarate, and pyruvate. Curiously enough malonate was found to have a similar effect (142), although in higher concentrations malonate inhibits growth. Two phenomena (besides the effect on oxidation) are to be distinguished,—the prevention or reversal of iodoacetate inhibition, and the actual promotion of growth. In a more extensive study Thimann & Bonner (144) made this distinction clear and showed that malonate does not increase the growth of coleoptile sections appreciably above that of controls, although it does prevent the iodoacetate inhibition. Inhibition by arsenite and phenyl-mercuric salts was not prevented by organic acids, and it was tentatively deduced (145) that these inhibitors may react at the same locus as iodoacetate but with a greater affinity for the enzyme. This locus is probably a sulfhydryl group. The question of whether

this is the same locus as that at which the organic acids themselves act is an interesting one for future study.

In addition to the acids mentioned above, isocitric, citric, and acetic were also studied (144). All these increased the growth by amounts up to 100 per cent. The growth promotion was not caused by promoting sugar uptake, since it occurred in the absence of sucrose. Of considerable interest is the related finding of Berger & Avery that dehydrogenases for citric, isocitric, aconitic, malic, and glutamic acids, as well as fumarase and aconitase, are present in the coleoptile (125, 134), although none of these shows activation by auxin. Spinach leaves also contain some of these enzymes (146). That coleoptile respiration proceeds by way of the Krebs cycle has been made very probable by Bonner (123), who showed, inter alia, that malonic acid causes the accumulation of succinic acid (see section on *Respiration*). This, of course, is prima facie evidence for the functioning of succinic dehydrogenase which Berger & Avery (134) could not detect by the Thunberg technique. That fumarate, malate, and succinate increase the respiration of coleoptiles was confirmed recently by Kelly & Avery (147).

The action of organic acids is smaller when the sections are cut from older coleoptiles whose growth is slowing down. This variation of effect with age can be correlated with changes in the organic acid content. More particularly, sensitivity to iodoacetate increases markedly with age of the coleoptile (144). The malate, citrate, and total organic acids decrease steadily over the same age range (148) which is interpreted as meaning that the organic acids are natural protective agents against iodoacetate. It appears (148) that the growing stems of *Pisum* seedlings have a content of total acids, citrate and malate even higher than that of young coleoptiles.

These experiments have also given evidence for a rôle of acetate in coleoptile growth (149). In presence of fluoroacetate, a specific inhibitor of acetate metabolism, the rapid growth which occurs under conditions of good aeration could be reduced to a low value close to that found with sections submerged in the test solution. The inhibition was largely removed by acetate. Since pea stems show a similar inhibition and counter-inhibition effect, their growth would also seem, at least partly, to involve acetate. A summary of some of the above work is given in (148).

The growth of other tissues is also stimulated by organic acids. Hildebrandt & Riker (150) found that tissue cultures of some species showed growth promotion by organic acids (sodium salts) in presence of 2 per cent sucrose. Growth of marigold tissue was promoted by succinic, malic, fumaric, and tartaric, Paris daisy tissue by succinic, and sunflower tissue by succinic and gluconic acids. Periwinkle and tobacco showed only inhibitions. However, since the acids were all tested at one rather high concentration (0.5 per cent, or about 0.03 to 0.04 M) a more systematic study of each might reveal other effects. The favorable influence of succinic, and the inhibitory effects of formic, acetic, propionic, lactic, glycolic, oxalic, glutaric, and citric acids were general. None of the acids could be substituted for sucrose as a carbon source.

In this connection it is of interest that *Phytomonas* tumors of beet con-

tain more oxalic, citric, and ascorbic acids than normal tissue (151). The normal tissue had an R.Q. of 0.64, which Neish & Hibbert ascribe to organic acid formation, while the tumor tissue gave 0.92. It is conceivable, therefore, that the more rapidly-growing tumor tissue consumes malate as fast as it is formed.

Whole seedlings show effects similar to the above, though the results reported are not as clear-cut as with Avena and include some apparent conflicts. With *Phaseolus aureus*, when growth was followed by root elongation, Blagoveshchenskii & Kolognivova (152) found that succinic acid stimulated growth at concentrations from 1.25×10^{-4} to 10^{-3} M. Fumaric and aspartic acids also stimulated up to 5×10^{-4} M but at 10^{-3} M they depressed the growth. Oxalic acid was inhibitory and at 10^{-3} M stopped growth completely.

In the previously mentioned study on roots, Nance (129) found that the uptake of nitrate by wheat roots was inhibited by 2,4-D, but that this inhibition was largely prevented or reversed by citric acid. The parallelism with the experiments on coleoptiles is very suggestive, although of course cell enlargement and salt uptake may be independent processes. While organic acids may reverse inhibitions, in general they do not necessarily promote growth of roots, for Lundegårdh (153) found [*cf.* (152)] that fumaric acid $(3 \times 10^{-5}M)$ as well as malonic acid, inhibited salt absorption and bleeding of wheat roots. The dependence of this response on pH, Lundegårdh ascribes to the effectiveness of only the undissociated acid (*cf.* section on *Respiration*). In a metabolic study of excised tomato roots, Henderson & Stauffer (130) showed that the 4-carbon acids, as well as pyruvate and citrate, increase the oxygen consumption in presence of sucrose, although they could not be used as substitutes for sucrose in growth.

With *Cattleya* seedlings grown on Knudson's medium, Withner (154) records that addition of pyruvic or malic acid at 2 mg. per liter (=ca. 2 $\times 10^{-5}M$) produced larger seedlings with darker green color and longer roots than the controls. It is more difficult to interpret the results of Pontovich (155) with Nausaghyz seedlings because of the pH changes involved. When potassium salts of various organic acids were added to the modified Knop's solution (pH 7.5) in which the plants were grown in sterile culture, the media became alkaline. Ammonium salts in the same concentration $(2 \times 10^{-3}M)$ made the medium acid. Thus the anions were taken up preferentially to the K' but less rapidly than the NH$_4$'. Succinate was the only anion which promoted growth both as a potassium and as an ammonium salt. However, it seems that, as with the data previously discussed, experiments at a single concentration, particularly when it is above $10^{-3}M$, are apt to give complex results. For a substance which is highly active, 10^{-3} M may well be a somewhat high concentration, as the reviewers have found in experiments with Avena. An inhibiting effect at a high concentration may not be in conflict with a stimulating effect at a low concentration.

In studying the growth of Guayule (*Parthenium argentatum*) Bonner & Galston (156) noted that plants on the periphery of an experimental plot

grew better than those in the center. Analysis of sand cultures showed that the roots excreted a substance which inhibited the growth of guayule seedlings. Similar inhibitory effects were given by water infusions of the roots, from which succinic and cinnamic acids could be isolated. Addition of cinnamic acid to seedlings in vials caused very marked growth inhibition. After 14 days in soil, however, the inhibition was no longer produced, indicating that the acid was decomposed by the microflora (157). Soil from around guayule plants did not inhibit guayule seedlings either, so that the original observation is not explained. The inhibition by cinnamic acid is nevertheless of some interest because of the relation between this compound and its geometrical isomer, *cis*-cinnamic acid, which has activity as an auxin. Auxins in general inhibit root elongation [*cf.* (158)]. Ordinary or *trans*-cinnamic acid was reported by Haagen-Smit & Went (159) and Koepfli, Thimann & Went (160) to be inactive as an auxin, i.e. in promoting growth, but no growth inhibiting effect was found. Blagoveshchenskii & Kolognivova (152) did find *trans*-cinnamic acid to inhibit growth of *Phaseolus* roots at concentrations above 2.5×10^{-4} M, though below this level some growth stimulation was observed. The isolation of *trans*-cinnamic acid from guayule rubber, resin, and leaves by Walter (161) is of interest in connection with the above observations; most of the acid was in the form of the parthenyl ($C_{15}H_{25}$) ester, but there were indications that some occurs free.

OCCURRENCE AND DISTRIBUTION

There are so many miscellaneous observations on the occurrence of organic acids that it has been thought best to present the data in tabular form. This should, it is hoped, provide a useful index to the scattered literature. The majority of the references are additional to those dealt with in the text. Data for tissues refer to dry weight unless otherwise stated.

CITRIC ACID

Plant	Material	Remarks	Reference
GRAMINEAE			
Sugar cane	juice	Small amount present; increases during sprouting	(162)
Lolium perenne	leaf clippings	0.96 to 0.50 per cent of total; decreases with time	(163)
Oat, barley, wheat and rye	seeds	0.01 to 0.03 of dry wt.; increases 2 to 6-fold during germination	(164)
Zea mays	seeds	0.20 per cent; little increase during germination	(164)
MAGNOLIACEAE			
Schizandra chinensis	fruit	Main acid	(165)
CRASSULACEAE			
Bryophyllum calycinum	leaves	2 per cent of the dry wt.	(52, 53)
Sedum acre	leaves	Present	(166)

Plant	Material	Remarks	Reference
ROSACEAE			
Apple (6 varieties)	fresh juice	*ca.* 3.6 m. eq. per liter; forms 3 to 10 per cent of total acid	(105)
Pear (17 varieties)	fresh juice	From 2 to 116 m. eq. per liter	(106)
Dewberry (2 hybrids)	fruit	85 per cent of total acid	(167)
Blackberry (3 varieties)	fruit	Contains none	(167)
LEGUMINOSAE			
9 species	seeds	0.33 (*V. faba*) to 2.14 per cent (*Phaseolus multiflorus*) of dry wt.	(164)
RUTACEAE			
Lemon	whole fruit	4.2 to 5 per cent of juice	(168)
	peeled fruit	7 to 7.5 per cent of juice	
Monghyr lemon		4.9 per cent of juice	(169)
Lemon		45.3 to 65.0 mg. per cc. of whole juice	(170)
Lemon, orange, and grapefruit	peel sap	0.19 to 1.22 mg. per cc. of sap	(171)
	dried peel	0.19 to 0.51 mg. per gm.	(171)
Orange	juice	Varies inversely with atm. temp.	(172)
Orange (Valencia)	leaves	12.3 per cent of ether soluble acid	(173)
Limes; *C. medica* and *C. decumana*	juice	Prepn. as calcium citrate described	(174)
Grapefruit	juice	20 to 23 mg. per cc.; over 90 per cent as free acid, which becomes partly neutralized during growth.	(175)
VITACEAE			
Grape	(Baden Wines)	431 mg. per liter	(176)
MALVACEAE			
Cotton	raw fibers	0.05 to 0.10 per cent	(177)
STERCULIACEAE			
Cacao	bean	0.45 to 0.64 per cent of dry wt.; oxalic the only other acid.	(178)
ERICACEAE			
Vaccinium		Among the best sources in U.S.S.R.	(179)
SOLANACEAE			
Potato (Green Mountain)	juice	20 times as concentrated as malic	(180)
Potato	leaves	0.882 to 1.280 per cent dry wt.	
	stems	0.165 to 0.149 per cent dry wt.	(181)
	tubers	0.812 to 0.966 per cent dry wt.	
Potato (Swedish; stored)	tubers	0.08 to 0.55 per cent fresh wt.	(182)
Potato, Swedish	tubers	0.5 to 1 per cent fresh wt.; commercial production described	(183)

Plant	Material	Remarks	Reference
Potato, nightshade, and bittersweet	leaves	2.85 to 4.95 per cent dry wt.	(179)
Tomato	"parts"	4 per cent maximum	(179)
Tobacco	waste	Extraction method	(184)
OTHER			
Makhorka		Extraction method	(185)
12 crop plants (greenhouse grown)	leaves, stems, and petioles)	Detailed analyses given	(186)

MALIC ACID

GRAMINEAE			
Wheat and blue grass	leaves	8 to 18 m. eq. per cent	(186)
Sugar cane	juice	0.000077 per cent by volume	(162)
Lolium perenne	leaf clippings	3.5 to 1.2 per cent of total; decreases with age	(163)
CHENOPODIACEAE			
Beet and spinach	leaves and petioles	Detailed analyses given	(186)
POLYGONACEAE			
Buckwheat	leaves and stems	Detailed analyses given	(186)
MAGNOLIACEAE			
Schizandra chinensis	fruit	A principal acid	(165)
CRASSULACEAE			
Bryophyllum calycinum	leaves	About 7 per cent of the dry wt. lower in p.m. than in a.m.	(50)
Sedum acre		Present	(166)
ROSACEAE			
Apple (6 varieties)	juice	35 to 147 m. eq. per liter; comprises 90 to 95 per cent of total acid; decreases with ripening	(105)
Apple	syrup	Method of production	(187)
	treacle	Method of production	(188)
Pear (17 varieties)	fresh juice	26 to 218 m. eq. per liter	(106)
Dewberry	fruit	8 to 11 per cent of total organic acids	(167)
Blackberry (3 varieties)	fruit	From 15 to 35 per cent of total organic acids	(167)
LEGUMINOSAE			
Pea, lima bean, soybean and alfalfa	leaves and stems	Detailed analyses given	(186)
RUTACEAE			
Lemon	juice	1.5 to 4.3 mg. per cc.	(170)
Lemon, orange, and grapefruit	peel sap dried peel	0.94 to 2.82 mg. per cc. 0.94 to 2.88 mg. per gm.	(171)
Orange (Valencia)	leaves	30.8 per cent of ether soluble acids	(173)
Grapefruit	juice	1.6 to 4.0 mg. per cc.	(175)

Plant	Material	Remarks	Reference
ANACARDIACEAE			
Rhus typhina	fruit	Calcium malate crystals in seed epidermis: 1.7 per cent of seed weight	(189)
VITACEAE			
Grape (California) (75 var.)	juice	Decreases with ripening	(190)
Grape		Decreased strongly during hot weather	(103)
Grape (Concord)	juice	0.22 to 0.44 gm. per 100 cc.	(191)
Grape (Southern Bulgarian Wine)		White: 3.766 gm. per liter Red: 1.720 gm. per liter	(192)
Grape (Hungarian Wines)		Methods and yields given; they vary widely	(193)
MALVACEAE			
Cotton	raw fiber	0.32 to 0.57 per cent	(177)
UMBELLIFERAE			
Carrot	fermented juice	0.9 per cent of total acid	(194)
SOLANACEAE			
Potato (Green Mountain)	juice	1/20th concentration of citric	(180)
Tobacco	waste	Extraction method	(184)
Tomato	leaves	31 to 61 m. eq. per cent	(186)
	stems	53 to 89 m. eq. per cent	(186)
CUCURBITACEAE			
Cantaloupe melon	leaves	22 to 26 m. eq. per cent	(186)
	stems	54 m. eq. per cent	(186)
COMPOSITAE			
Lettuce	leaves	72 to 109 m. eq. per cent	(186)

OXALIC ACID

Plant	Material	Remarks	Reference
BRYOPHYTES			
Funaria and *Sphagnum*	leaves	Calcium oxalate crystals present	(195)
GRAMINEAE			
Sugar cane	juice	0.00004 per cent by volume	(162)
Lolium perenne	leaf clippings	0.31 to 0.20 per cent of total; decreases with age	(163)
Blue grass and wheat	leaves	Contain none	(186)
CHENOPODIACEAE			
Spinach	leaves	208 to 309 m. eq. per cent	(186)
Spinach	leaves	1 part in 313	(196)
Spinach	petioles	110 to 151 m. eq. per cent	(186)
Spinach (31 varieties)	leaves	500 to 800 mg. per 100 gm. dry wt.; only about 1/3 as calcium salt	(197)
Halogeton glomeratus	whole plant	Up to 20 per cent of total dry wt., in fall	(198)

Plant	Material	Remarks	Reference
Beta vulgaris (red beet)	leaves	High content (ca 0.3 per cent)	(199)
Beta vulgaris	leaves	298 to 323 m. eq. per cent	(186)
	petioles	87 to 98 m. eq. per cent	(186)
AMARANTHACEAE			
Alternanthera sessilis	leaves	6.8 per cent of dry wt.	(200)
Amaranthus aquatica		0.383 per cent of fresh wt.	(199)
Amaranthus polygonoides	leaves	11.25 per cent of dry wt.	(200)
Amaranthus gangeticus	leaves	6 per cent of dry wt.	(200)
PORTULACACEAE			
Talinum speciosa	leaves	12.7 per cent of dry wt. (in spite of being eaten as a vegetable in Ceylon)	(200)
CRUCIFERAE			
Brussels sprouts		Deficient in oxalates	(196)
LEGUMINOSAE			
Pea, lima bean, alfalfa	leaves	14 to 30 m. eq. per cent	(186)
and soybean	stems	5 to 31 m. eq. per cent	(186)
Cassia tora	leaves	0.26 per cent of dry wt.	(200)
Selbania grandiflora	leaves	0.8 per cent of dry wt.	(200)
RUTACEAE			
Lemon, orange, and grapefruit	dried peel	0.50 to 1.80 mg. per gm.	(171)
Valencia orange	leaves	39.9 per cent of ether soluble acids	(173)
VITACEAE			
Grape	vine "shoots"	Most abundant acid	(201)
MALVACEAE			
Cotton	dried root bark	Electrodialysis yields 5 gm. per kg.	(202)
Cotton	raw fibre	0.002 to 0.005 per cent of dry wt.=0.3–0.6 per cent total acid	(177)
TERNSTROEMIACEAE			
Black tea	leaves	1 part in 270 of dry wt.	(196)
Black tea	leaves	1.24 per cent of dry wt.	(200)
STERCULIACEAE			
Cacao	bean	0.32 to 0.50 per cent of dry wt.	(178)
UMBELLIFERAE			
Carrot	fermented juice	1.0 per cent of total acid	(194)
SOLANACEAE			
Potato (Green Mountain)	juice	0.02 per cent	(180)
Tomato	fruit	less than 1 part in 20,000	(196)
	leaves	45 to 69 m. eq. per cent	(186)
	stems	82 m. eq. per cent	(186)
Tobacco	waste	method of extraction	(184)
COMPOSITAE			
Lettuce	leaves	1.4 to 2.3 m. eq. per cent	(186)

Plant	Material	Remarks	Reference
OTHER			
"Malaya vegetables"		Oxalate content often high [cf. also (200)]	(203)
"Formosa vegetables"		Oxalate content given	(199)

ISOCITRIC ACID

CRASSULACEAE (Crassulacean malic identified as isocitric acid)			(50, 51, 166)
Bryophyllum calycinum	leaves	At least 8 per cent of the dry wt.	(50)
Sedum acre and *Echeveria secunda*		Present in place of "crassulacean malic"	(166)
ROSACEAE			
Dewberry (2 hybrids)		5 per cent of total organic acids	(167)
Blackberry (3 varieties)		Constitutes 65 to 85 per cent of total organic acids	(167)
SOLANACEAE			
Potato (Green Mountain)	juice	Small amount present	(180)

SUCCINIC ACID

GRAMINEAE			
Sugar cane	juice	Small amount present	(162)
CRASSULACEAE			
Sedum acre	leaves	present (by isolation)	(166)
ROSACEAE			
Apple	juice	1.5 per cent of total acid	(105)
VITACEAE			
Grape (Southern Bulgarian wines)		White: 0.524 gm. per liter Red: 0.814 gm. per liter	(192)
Grape (Hungarian wines)		Methods and yields given; content somewhat higher than in wines of other countries	(193)
UMBELLIFERAE			
Carrot	fermented juice	7.2 per cent of total acid	(194)
OTHER			
Calabash curare	powder	present (by isolation)	(204)

TARTARIC ACID

MAGNOLIACEAE			
Schizandra chinesis	fruit	A principal acid	(165)
LEGUMINOSAE			
Tamarindus indica	fruit	10.8 per cent of dry wt., as free acid, plus 6.4 per cent as potassium acid salt	(205)

Plant	Material	Remarks	Reference
VITACEAE			
Grape (California) (75 var.)		Decreases with ripening but less so than malic	(190)
Grape		Decreases during hot weather but less so than malic	(103)
Grape	juice	Becomes esterified as juice is concentrated	(206)
Grape (Concord)	juice	0 to 0.26 gm. per 100 cc.	(191)
Grape (Southern Bulgarian wines)		White: 2.041 gm. per liter Red: 1.230 gm. per liter	(192)
Grape (10 Hungarian wines)		Content somewhat lower than in wines of other countries	(193)

PYRUVIC ACID

Plant	Material	Remarks	Reference
LILIACEAE			
Onion (Ebenezer)		May accumulate and disappear (no analysis given)	(207)
Onion	juice	0.103 gm. per 100 cc.	(208)

CHLOROGENIC ACID

Plant	Material	Remarks	Reference
CONVOLVULACEAE			
Sweet Potato	root	*ca.* 2 gm. from 50 lbs.	(209)
SOLANACEAE			
37 species	leaves and other parts	Present in all. Distribution in 3 species studied microscopically; strongest in fruit and placenta; none in embryos. Typically in epidermis	(210)
COMPOSITAE			
species from 95 genera		Present in all, especially in epidermis and cells adjoining leaf-veins	(211)

LACTIC ACID

Plant	Material	Remarks	Reference
ROSACEAE			
Apple (6 varieties)	fresh juice	0.3 to 0.8 m. eq. per liter; comprises about 1.5 per cent of total acid	(105)
VITACEAE			
Grape (Southern Bulgarian wines)		White: 0.920 gm. per liter Red: 2.160 gm. per liter	(192)
Grape (Hungarian wines)		Method and yields given	(193)
UMBELLIFERAE			
Carrot	fermented juice	67 per cent of total acid	(194)

ACETIC ACID

Plant	Material	Remarks	Reference
VITACEAE			
Grape (Southern Bulgarian wines)		White: 0.432 gm. per liter Red: 0.768 gm. per liter	(192)

TANNIC ACID

GRAMINEAE			
Sugar cane	juice	Small amount present	(162)
VITACEAE		•	
Grape (Hungarian wines)		Method and yields given	(193)

OTHER ACIDS

BROMELIACEAE			
Pineapple	fruit	Saccharinic acid 0.38 per cent by weight of total acids present, determined by isolation of lactone	(58)
GRAMINEAE			
Sugar cane	juice	Aconitic 0.05 per cent by vol.; glycolic, smaller amount	(162)
	evaporated juice	trans-aconitic 0.75 to 1.33 gm. per 100 gm. solids	(212)
PLATANACEAE .			
Plane Tree (*P. acerifolia*)	bark	Betulinic acid, $C_{30}H_{48}O_3$, isolated; 7 gm. per kg.	(213)
LEGUMINOSAE			
Pea	whole plants	Oxaloacetic 30 to 40 mg. per kg. wet wt. at noon; decreases in darkness	(35)
Red clover	whole plants	Oxaloacetic 100 mg. per kg. wet wt. at noon	(35)
DICHAPETALACEAE			
Dichapetalum cymosum ("*gifblaar*")		Monofluoroacetic acid found to be toxic principle; glycolic acid also isolated	(214, 215)
COMPOSITAE			
Guayule	root infusion	Trans-cinnamic	(156)
	rubber	Parthenyl cinnamate *ca.* 20 per cent of acetone-soluble	(161)
	leaves	Cinnamic present, partly free, partly as parthenyl ester	(161)
OTHER FAMILIES			
Calabash curare	powder	Protocatechuic and mesaconic acids isolated	(204)

TOTAL ACIDS

Plant	Material	Remarks	Reference
GRAMINEAE			
Sugar cane	juice	Increase when canes sprout	(162)
Lolium perenne	leaf clippings	100 m. eq. per 100 gm. dry wt.; no decrease with age	(163)
Wheat	leaves	122 to 133 m. eq. per cent	(186)
Blue grass (*Poa* sp.)	leaves	108 to 143 m. eq. per cent	(186)
POLYGONACEAE			
Buckwheat	leaves	354 to 360 m. eq. per cent	(186)
	stems	206 m. eq. per cent	(186)
CHENOPODIACEAE			
Beet	leaves	406 to 427 m. eq. per cent	(186)
	petioles	202 m. eq. per cent	(186)
Spinach	leaves	362 to 380 m. eq. per cent	(186)
	petioles	226 to 238 m. eq. per cent	(186)
MAGNOLIACEAE			
Schizandra chinensis	fruit	8.5 per cent acids, mainly citric, malic, and tartaric	(165)
CRASSULACEAE			
Bryophyllum calycinum	leaves	See section on DETERMINATION AND SEPARATION	(50)
ROSACEAE			
Apple	juice	See under CITRIC and MALIC ACID	(105)
Pear	juice	See under CITRIC and MALIC ACID	(106)
LEGUMINOSAE			
Lima bean, pea, alfalfa and soybean (greenhouse)	leaves	186 to 256 m. eq. per 100 gm. dry wt.	(186)
Lima bean, pea, alfalfa and soybean (greenhouse)	stems	141 to 259 m. eq. per 100 gm. dry wt., usually less than in leaves	(186)
RUTACEAE			
Lemon	juice	45.6 to 70.1 mg. per cc.	(170)
Lemon, orange, and grapefruit	peel	Less than in pulp	(171)
Lemons, tangerines, oranges, limes and pomelos		Correlated with vitamin C content	(216)
Orange (Valencia)	leaves	All soluble in water except oxalic	(173)
Grapefruit		Free acid falls and pH rises as fruit enlarges	(175)
VITACEAE			
Grape (75 California varieties)		Change with ripening	(190)

Plant	Material	Remarks	Reference
Grape (Hungarian wines)		Methods and yields given	(193)
MALVACEAE			
Cotton	raw fiber	0.77 to 0.91 per cent	(177)
STERCULIACEAE			
Cacao	bean	All accounted for as oxalic and citric	(178)
SOLANACEAE			
Tomato	leaves	240 to 270 m. eq. per cent	(186)
	stems	220 to 232 m. eq. per cent	(186)
CUCURBITACEAE			
Cantaloupe melon	leaves	86 to 99 m. eq. per cent	(186)
	stems	119 to 130 m. eq. per cent	(186)
COMPOSITAE			
Lettuce	leaves	197 to 221 m. eq. per cent	(186)

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TRANSFORMATION OF SUGARS IN PLANTS

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The first sugar of photosynthesis.—Glucose, fructose, sucrose, and starch are formed in green plants as a result of photosynthetic activity and can easily be detected. Because of this, plant physiologists devoted considerable effort for over half a century to prove which of these carbohydrates constitutes the "first sugar of photosynthesis." Their experimental approach was to determine the relative concentrations of these carbohydrates in the plant at different times of the day and seasons of the year and to study the changes caused by respiration and illumination. On the basis of such experiments each of the carbohydrates mentioned, especially sucrose and glucose, has been claimed by various investigators to be the first photosynthetic product. However, they neglected to take into consideration the rapidity with which monosaccharides formed in the process of photosynthesis can be isomerized and polymerized to complex carbohydrates by the enzymes in the leaves, and the speed with which the complex carbohydrates can be broken down to monosaccharides by the reverse reactions. The preponderance of a particular sugar at a certain time cannot be interpreted to mean that it is the first to be formed. The subject of carbohydrate transformation has been excellently summarized by several workers in the field (1).

It was established within recent years that the mechanism of the photosynthetic process (2) consists of a series of reactions which succeed each other so closely that the intermediate products formed are not detectable by common analytical methods. When a green leaf is illuminated, glucose, fructose, sucrose, and starch rapidly appear; however, light and chlorophyll are not required for their formation. That light is not necessary for the starch synthesis is shown by the fact that it can be formed *in vitro* in the dark from glucose-1-phosphate (Cori ester) by means of the enzyme phosphorylase (3). Likewise, the process of sucrose formation does not depend on light. Hartt (4) demonstrated that synthesis of sucrose from glucose and fructose in detached sugar cane blades takes place in the dark and in the absence of chlorophyll. Similar results were obtained by McCready & Hassid (5) with barley shoots which were infiltrated with monosaccharides, indicating that the synthesis of sucrose is a process distinct from photosynthesis. Since the snow plant, *Sarcodes sanguinea*, which is devoid of chlorophyll and derives its nutrients from organic matter in the soil, contains a considerable quantity of glucose and fructose (6), it is evident that light is not required for the formation of these monosaccharides. It therefore appears that light is required only for the reaction involving the initial phases of carbon dioxide utilization in the photosynthetic process. When the primary organic molecules are produced, sugars, including monosaccharides, can subsequently be

formed in the absence of light. In view of these facts, the older term "first sugar of photosynthesis" has no significant meaning. Nevertheless, the question as to whether monosaccharides precede sucrose formation or vice versa is still of biochemical interest.

Smith (7) showed that sunflower leaves supplied with carbon dioxide formed sucrose and starch immediately upon the beginning of illumination, while the increase in the quantity of the monosaccharides was initially relatively small and increased with time. From these observations he concluded that free monosaccharides found in the plant cells are secondary products, produced by the hydrolysis of sucrose.

Calvin & Benson's (8) results with *Chlorella pyrenoidosa* also indicate that sucrose synthesis precedes monosaccharide formation. Experimenting with algae, the plants were allowed to photosynthesize in the presence of C^{14}-labeled sodium bicarbonate for short periods of time. They were then extracted and the photosynthetic products separated on a paper chromatogram, which served as a semiquantitative record of the activity fixed in each compound. In the short photosynthetic experiments (30 to 90 sec.) the major portion of the newly reduced carbon dioxide was found in the phosphoglyceric acids, triose phosphates and the hexose phosphates. The first free carbohydrate which appeared in these plants was sucrose. Glucose and fructose also appeared on the chromatogram, but they were not radioactive. These authors maintain that if free glucose or free fructose were intermediates in the synthesis of sucrose, they should have become radioactive either prior to the appearance of radioactive sucrose or simultaneously with it. Calvin & Benson, therefore, suggested that the immediate sucrose precursors are probably glucose-1-phosphate and fructose-6-phosphate, and that when sucrose is formed through phosphorolytic condensation the phosphate is simultaneously split off. The hexosephosphates are presumably synthesized from triose phosphates.

However, there is an abundance of experimental evidence to show that plants can readily form sucrose from hexose monosaccharides (4,5,9, to 12). Assuming that sucrose is always synthesized from the same immediate phosphorylated hexose precursors, it would appear that in the experiments where the disaccharide is formed from free monosaccharides, the latter must be converted to the same hexosephosphates as those synthesized through a different path (possibly via phosphorylated triose phosphates as suggested by Calvin & Benson's work with Chlorella).

Interconversion of monosaccharides.—It has been demonstrated by a number of investigators that plant cells possess an enzymatic system capable of interconverting monosaccharides. Thus, Nelson & Auchincloss (9) showed that sucrose can be synthesized *in vivo* in the potato at the expense of glucose or fructose. That such a conversion also occurs in other plants has been demonstrated by Virtanen & Nordlund (10) who worked with red clover and wheat plants, and by Hartt (11) with detached sugar cane leaves.

Nurmia (12) made a detailed study of the transformations that various sugars undergo in living plant tissue, independently of the photosynthetic

process. The experiments were carried out with wheat, oats, clover, and broad bean. The plants were first placed in the dark from 24 to 48 hr. to reduce the sugar to a low level, then cut and the stems or petiols placed in aqueous 10 per cent sugar solutions for another 24 hr. in the dark. The leaves and stalks were analyzed separately. The data showed that glucose and fructose are readily converted into each other in living plant tissues and that the interconversion of hexose sugars was always accompanied by a considerable synthesis of sucrose. In some cases as much as 50 per cent of each of the hexoses taken up by the plants was interconverted and then changed into sucrose. Nurmia demonstrated that sucrose could be synthesized from D-glucose, D-fructose, D-galactose and maltose, but not from dihydroxyacetone, D-xylose, or glycerine. The interconversion of the hexose sugars took place in the stem, which also appeared to be the seat of sucrose synthesis. In the leaves the changes in the different sugar fractions were generally less marked than in the stem.

McCready & Hassid (5), working with barley plants, infiltrated the following compounds: D-glucose, D-fructose, D-mannose, D-galactose, lactose, maltose, L-arabinose, D-xylose, mannitol, sorbitol, gluconic acid, pyruvic acid, and glyceric acid. The barley leaves were cut from the plant and allowed to respire in the dark for 24 hrs. to deplete them of sucrose. They were then placed either in water or in 10 per cent solution of the particular sugar to be studied and subjected to vacuum infiltration. This procedure consists of submerging the tissue in the desired solution in a container such as a desiccator which can be evacuated. During evacuation the gas is removed from the intercellular spaces of the tissue, and on release of the vacuum the solution in which the leaves are immersed is drawn into the tissue. Intimate contact between the cells of a plant tissue and any desired solution may thus be achieved. After infiltration the barley leaves were removed from the solution and allowed to remain for a certain period of time in the dark in an atmosphere saturated with water vapor. The plant material was then thoroughly washed and analyzed for sugars.

The data showed that in leaves not supplied with any of the sugars, the sucrose disappeared completely within about 24 hrs. However, in leaves supplied with glucose or fructose, sucrose accumulated to a level of about 6 per cent of the dry weight. Smaller accumulations of sucrose were obtained at the expense of galactose, mannose, and glyceric aldehyde.

The fact that maltose and lactose were also utilized by barley for sucrose formation indicates that the plants apparently contain enzymes which are able to hydrolyze these disaccharides to their respective monosaccharides, which are then used for the synthesis of sucrose. The pentose sugars, L-arabinose and D-xylose, were not utilized for sucrose formation. Neither mannitol, sorbitol, nor gluconic acid was used by the plant for sucrose formation, indicating that barley contains no enzymes which could oxidize the sugar alcohol or reduce the sugar acid. It has been observed that when oxygen is eliminated during the incubation of the monosaccharide-infiltrated plants, synthesis of sucrose does not occur, showing that the process is aerobic. The

observation that sucrose is not synthesized from glucose in potato slices in the absence of oxygen was previously made by Nelson & Auchincloss (9).

It is a well known fact that potatoes, when stored at cold temperature, convert their starch to sucrose. However, McCready (13) found that this conversion will not take place in the absence of oxygen or in the presence of cyanide. This observation confirms the previous finding (9) that the process of sucrose formation is apparently aerobic. During the process of starch conversion hexosemonophosphates and fructose-1,6-diphosphate accumulated. McCready assumes that the latter compound is probably an essential component for sucrose formation.

In studying the changes in composition of potato tubers that take place during storage at low and high temperatures, Arreguin-Lozano & Bonner (14) found that at the lower temperature the balance between glucose-6-phosphate and fructose-6-phosphate is in favor of the latter hexosephosphate. They also observed that the phosphorylase is equally active at all temperatures. The failure to attack starch at high temperatures is explained by the assumption that a phosphorylase inhibitor is formed which disappears at low storage temperature.

Extensive investigations on the interconversion of glucose and fructose to sucrose in the sugar cane leaf have been carried out by Hartt (4, 11) at the Hawaiian Islands Pineapple Experiment Station. She showed that synthesis of sucrose from glucose can take place in all parts of the plant, *i.e.*, in the detached blades, sheaths, stems, and in the entire stalks. The detached blades of the sugar cane plant can continue to synthesize sucrose from glucose for approximately two weeks. During this period there is a steady increase from an initial 3 per cent to about 16 per cent cane sugar on the dry basis.

Since sucrose is synthesized from glucose or fructose in the dark in etiolated blades, its process of synthesis can be considered nonphotosynthetic. Hartt also demonstrated that etiolated shoots, when supplied with glucose, accumulated fructose and when supplied with fructose, accumulated glucose, showing that the shoots possess a mechanism for mutual interconversion of these monosaccharides. Her results further indicate that aeration is essential for the interconversion of glucose and fructose and for the formation of sucrose.

Mechanism of interconversion of monosaccharides.—The experimental data of various investigators (11, 12, 13) clearly show that glucose and fructose are interconvertible in plants. Since it has been demonstrated that galactose and mannose (12, 13) are also utilized by a number of plants for sucrose formation, it must be assumed that these monosaccharides are converted to glucose and fructose before such synthesis can occur. The question is, how does the interconversion of the monosaccharides take place? The mutual (nonenzymatic) interconversion of glucose, fructose, and mannose can be accomplished *in vitro* in alkaline solution through the Lobry-Bruyn enolic transformation. Spoehr & Strain (15) showed that such conversion can also take place in neutral or even slightly acid solution in the presence of inorganic

phosphate. However, there is no evidence that interconversion of these monosaccharides in plants takes place by a mechanism of enolization. The evidence rather points to the fact that the transformations occur through the mediation of enzymes involving phosphorylation reactions. It is known that glucose is transformed by yeast hexokinase to glucose-6-phosphate in the presence of the phosphate donor, adenosinetriphosphate (16). This energy-rich phosphate compound is found in animal and bacterial cells and has been recently reported to be present in a higher plant (mung bean) (17). Extracts of muscle, brain, liver, and yeast contain an enzyme, Lohmann's isomerase (18) which catalyzes the reaction: glucose-6-phosphate$\rightleftharpoons$fructose-6-phosphate. A mixture of the two esters was isolated by Robison from yeast and by Embden from muscle; these esters are also known to occur in higher plants (19). A phosphohexoisomerase capable of catalyzing the reversible interconversion of glucose-6-phosphate to fructose-6-phosphate and mannose-6-phosphate, was shown by Tankó (20) to exist in pea extracts. Somers & Cosby (21) also found that fructose-6-phosphate is converted by pea seed extracts into an aldose monophosphate, presumed to be glucose-6-phosphate. Hanes (22) also showed that when crude pea extracts are allowed to act upon starch in the presence of inorganic phosphate, the polysaccharide is converted into a mixture of reducing hexosephosphates of which a considerable proportion has been shown to consist of 6-phosphate esters of glucose and fructose. The free hexoses are probably formed as a result of phosphatase activity by splitting off the phosphate from the hexosemonophosphates. Phosphatases capable of hydrolyzing phosphoric acid esters are widely distributed in plants (23).

Information concerning the mechanism of conversion of galactose to glucose or fructose is not available, but it is conceivable that similar phosphoisomerases operate in plants which make this transformation possible.

In connection with the mechanism involved in the transformations of phosphorylated intermediates, Stumpf (24) isolated an enzyme, aldolase, from pea seeds that catalyzes the following reversible reaction:

$$\text{Fructose-1,6-diphosphate} \rightleftharpoons \begin{matrix} \text{dihydroxyacetone} \\ \\ \text{phosphate} \end{matrix} + \text{D-3-glyceraldehyde phosphate}$$
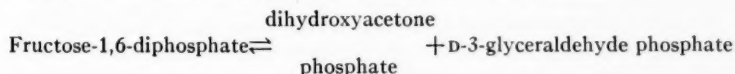
Tewfik & Stumpf (25) further demonstrated that aldolase is widely distributed in plants. Analysis of 29 different plants (fungi, ferns, conifers, monocotyledons, and dicotyledons) showed that all of them had a measurable aldolase activity. It was therefore concluded that this enzyme plays a central rôle in the transformation of sugars in plants. It is interesting to note that aldolase appears in highest concentration in actively growing parts of plant tissues and is localized in the cytoplasm of the leaf cells rather than in the chloroplasts.

In a series of experiments Stumpf (26) further determined the component enzymes involved in the fermentation system of pea seeds. Besides (a) aldolase, the following enzymes were shown to be present: (b) isomerase, (c) triose

phosphate dehydrogenase, (*d*) enolase, (*e*) phosphotransferase, and (*f*) carboxylase.

Isomerase was shown to be responsible for the reversible reaction: dihydroxyacetone phosphate$\rightleftharpoons$D-3-phosphoglyceraldehyde. Triosephosphate dehydrogenase oxidizes the D-3-phosphoglyceradehyde to phosphoglyceric acid. The components of this system include coenzyme I, arsenate, or phosphate, and an oxidant required to oxidize reduced coenzyme I. The function of enolase is to transform phosphoglyceric acid to phosphopyruvic acid. Phosphotransferase shifts phosphate from phosphopyruvic acid to adenylic acid generating adenosine diphosphate or adenosine triphosphate. Pyruvic acid carboxylase activates the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide.

Stumpf's systematic investigations of intermediary metabolism in plants undoubtedly constitute the best available evidence that the enzymatic reactions involved in the glycolytic system of plants are similar to those in yeast and animal tissues.

Mechanism of sucrose synthesis.—Since the sucrose splitting enzyme, invertase (β-fructofuranosidase), is almost universally distributed in plants, it was generally believed that this hydrolyzing enzyme was responsible for the conversion of sucrose to invert sugar in the living plant. It was, therefore, the breakdown rather than the synthesis of sucrose that first received attention. The assumption was also made that, under favorable conditions, the same hydrolyzing enzyme played some rôle in reversing the process, combining glucose with fructose into sucrose. It may be of interest to point out that the early investigations of the mechanism of the breakdown and synthesis of starch and glycogen with the enzyme, amylase, followed as an analogous course of events.

Invertase, like amylase, catalyzes the rupture of glycosidic linkages by the introduction of a molecule of water as follows:

$$>CH\!-\!O\!-\!HC< +H_2O = \,>CHOH +HOHC <$$
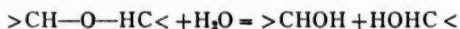
Because all enzymatic reactions are potentially reversible, it was reasonable to regard the synthesis of complex saccharides as the reverse of hydrolysis reactions. This assumption was supported to some extent by the observation that under special conditions other types of hydrolytic enzymes, e.g., the lipases, can catalyze the synthesis of appreciable amounts of glycerides and that emulsin (β-glucosidase) can synthesize glucosides from non-sugar residues and glucose. Some workers have produced evidence purporting to demonstrate that invertase catalyzes synthesis as well as hydrolysis of sucrose. Oparin & Kurssanov (27) claimed to have synthesized sucrose from invert sugar under the influence of invertase and phosphorylase in the presence of inorganic phosphate. However, careful repetition of this work by Lebedev & Dikanova (28) and other investigators failed to substantiate this claim.

When invertase is allowed to act upon a dilute solution of sucrose, the reaction in which invert sugar is formed goes almost to completion. Theo-

retically, when equilibrium is reached, a finite amount of sucrose must remain in solution, its concentration being determined by the free energy change of the reaction and the concentrations of the hydrolysis products. Since the ΔF for the hydrolysis of sucrose is approximately $-6,500$ calories per mole, the reaction has a strong tendency to go to the right. This tendency is greatly increased by the fact that one of the reactants is water and its concentration is enormously increased because the reaction takes place in aqueous medium. These factors are responsible for the fact that the reaction of sucrose hydrolysis is practically irreversible. From these considerations it may be concluded that hydrolytic enzymes are of no direct importance in the synthesis of sucrose, starch, and other complex carbohydrates.

Inasmuch as leaves of sucrose-producing plants, such as beets and peas (19, 20) were found to contain considerable amounts of hexose phosphates, it was suggested that sugar phosphates might be involved in the mechanism of sucrose formation and that phosphorylation is an essential step in this process (19b). That phosphorylation is essential for sucrose formation is also indicated by the fact that iodoacetate, which inhibits phosphorylation, also inhibits sucrose synthesis (29).

While this hypothesis has not as yet been supported by the isolation of an enzyme capable of sucrose synthesis from a plant source, Doudoroff, Kaplan & Hassid (30) prepared such an enzyme from a bacterial source. The dried cells from *Pseudomonas saccharophila* contain an enzyme which catalyzes the breakdown of sucrose in the presence of inorganic phosphate with the formation of D-glucose-1-phosphate and D-fructose. It was shown that this reaction is reversible, i.e., the same sucrose phosphorylase will catalyze the synthesis of sucrose from glucose-1-phosphate and fructose according to the reaction:

$$\text{D-glucosyl-fructoside} + \text{phosphate} \rightleftharpoons \text{D-glucose-1-phosphate} + \text{fructose}$$
$$\text{(sucrose)}$$
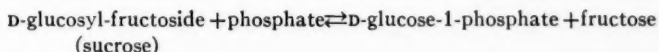
The crude enzyme preparation contains the hydrolytic enzyme, invertase, which competes with the sucrose phosphorylase for sucrose. However, most of the invertase can be eliminated from the bacterial preparations by ammonium sulfate fractionation. Using a partially purified sucrose phophorylase preparation and a mixture of glucose-1-phosphate and fructose, Hassid, Doudoroff & Barker (31) succeeded in crystallizing a nonreducing disaccharide which was identical with natural sucrose. Its structure is α-D-glucopyranosyl-β-D-fructosfuranoside.

The reaction involving the formation of glucose-1-phosphate and fructose from sucrose and inorganic phosphate is similar to the phosphorolysis of starch (3) and glycogen (32). It occurs as the result of phosphorolytic cleavage of glucose from the sucrose molecule, the sucrose being broken down without water entering into the reaction. The formation of sucrose from glucose-1-phosphate and fructose takes place by the reverse reaction as a consequence of "de-phosphorolytic" condensation of the two monosac-

charides. The reversible phosphorolysis of sucrose can be represented as shown in FIG. I.

The equilibrium constant for the reaction, expressed by the mass law equation,

$$K = \frac{\text{(sucrose) (inorganic phosphate)}}{\text{(fructose) (glucose-1-phosphate)}}$$

is 0.053 at pH 6.6 and 30°C.

At equilibrium, the catalyzed reaction favors the breakdown rather than the synthesis of sucrose. When glucose-1-phosphate and inorganic phosphate are present in equal concentrations in the equilibrium reaction mixture, the fructose concentration will be approximately twenty times that of the sucrose. From the equilibrium constant of the reaction it is possible to calculate the free energy change for the phosphorolytic reaction,

$$\Delta F^\circ_{303} = -RT\ln K = -1{,}385 \log 0.053 = 1{,}770 \text{ calories.}$$

Assuming that the free energy change of the reaction is entirely due to the difference in bond energies of sucrose and glucose-1-phosphate, the energy of the glycosidic bond of sucrose can be estimated. Since the energy of the C-O-P bond in glucose-1-phosphate is known to be 4,800 calories, the value
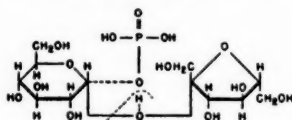
FIG. I. Phosphorolysis of Sucrose

for the glycosidic bond in sucrose must, therefore, be 4,800 cal. +1,700 cal. =6,500 calories, indicating that the hydrolysis of sucrose is exergonic to approximately this extent. The value obtained in this way is considerably greater than might have been anticipated and may help to explain the distinctive rôle of sucrose in various metabolic processes.

It should be pointed out that sucrose phosphorylase of *Pseudomonas saccharophila* is specific to the glucose portion of its substrates. The enzyme is incapable of combining with other hexosephosphates, such as α-D-galactose-1-phosphate, α-D-mannose-1-phosphate, α-D-xylose-1-phosphate or α-L-glucose-1-phosphate (33). On the other hand, the enzyme is far less specific with regard to substitutents for the second sucrose component, D-fructose. Several ketose monosaccharides, such as D-xyloketose, L-araboketose and L-sorbose, can replace fructose in the reaction with glucose-1-phosphate, giving rise to the corresponding nonreducing disaccharides, D-glucosyl-D-xyloketoside, (34), D-glucosyl-L-araboketoside (35) and D-glucosyl-L-sorboside (36). These disaccharides are analogues of sucrose in which the first carbon atom of the glucose is linked to the second carbon atom of the ketose. The enzyme can also cause a reaction between glucose-1-phos-

phate and the aldose, L-arabinose, to form a reducing disaccharide, D-gluco-syl-L-arabinose, having a 1,3-glucosidic linkage (37).

A similar enzyme system that would combine glucose-1-phosphate and fructose to form sucrose and inorganic phosphate has not yet been isolated from the tissues of higher plants. Although intact leaves or other intact tissues readily form sucrose from monosaccharides, preparations made from plants will not carry out the same reaction *in vitro*. Maceration or grinding of the tissue appears to inactivate the sucrose synthesizing mechanism. However, biochemical studies of various species of plants support the view that synthesis of sucrose may involve chemical reactions in which phosphate esters of glucose, fructose, or both hexoses serve as substrates, although the mechanism is probably not identical with that of the bacterial enzyme system.

Hartt (11c, 11d) found that certain poisons which are known to inhibit the formation of fructose diphosphate, are also active in preventing the formation of sucrose. However, when the breakdown of fructose diphosphate is inhibited, synthesis of the disaccharide is increased. This observation led her to conclude that sucrose diphosphate is probably an immediate precursor in the reaction of sucrose synthesis and suggested the following mechanism for its formation: glucose +fructofuranose diphosphate→sucrose phosphate →sucrose +phosphate. Hartt assumes the formation of an intermediary sucrose phosphate compound, which immediately splits into free sucrose and phosphate. However, such a scheme of sucrose synthesis is unlikely, because if sucrose is synthesized through dephosphorolytic condensation involving a hexosephosphate, it would be reasonable to assume that one of the components should be linked to the carbon atom having the potential reducing group. Glucose-1-phosphate or fructose-2-phosphate (hypothetical compound) would thus be expected to be involved in such synthesis.

Formation of sucrose and other disaccharides through exchange of glycosidic linkages.—Further studies (38) of the *P. saccharophila* sucrose phosphorylase system revealed that glucose-1-phosphate is not an essential product or substrate for the formation and breakdown of synthetic disaccharides. It was demonstrated that this ester could be considered merely as one of a number of "glucose-donors" for the enzyme. The enzyme itself was shown to be not only a "phosphorylase" but also a "transglucosidase," capable of mediating the transfer of the glucose of the substrates to a variety of "acceptors." Disaccharides could thus be synthesized and broken down through the exchange of one glycosidic linkage for another.

The glucose-transferring function of sucrose phosphorylase was discovered through the use of radioactive phosphate. When the enzyme, unlabeled glucose-1-phosphate and P^{32}-labeled inorganic phosphate were incubated together in the absence of fructose, no reaction was anticipated since one of the components of the phosphorylase system was missing. However, it was found after a short time that the glucose-1-phosphate became labeled with P^{32} and the labeled inorganic phosphate was diluted with unlabeled phosphate derived from the ester. This result can be satisfactorily explained only

by the assumption that the enzyme combines reversibly with the glucose portion of glucose-1-phosphate releasing inorganic phosphate in accordance with the equation:

$$\text{glucose-1-phosphate} + \text{enzyme} \rightleftharpoons \text{glucose-enzyme} + \text{phosphate}.$$

Since inorganic phosphate is released as the reaction goes to the right and is taken up in the reverse direction, the result of this reversible reaction is a transfer of glucose from one phosphate group to another. The transfer must occur in such a way as to preserve the energy of the glucosidic linkage; the exchange cannot involve the formation of free glucose, because if this occurred, 4,800 calories would be dissipated in the decomposition of the ester and would be required for its resynthesis. Inasmuch as no outside source of energy was available for the resynthesis of the ester, it must be concluded that the original bond energy is conserved in the above reaction.

On the basis of the theory that sucrose phosphorylase is a transferring enzyme, the glucose-enzyme complex should be capable of donating glucose to suitable acceptors other than phosphate. Indeed it was later shown (39) that in a phosphate-free medium, sucrose phosphorylase brings about the exchange of added free C[14]-labeled fructose with the fructose portion of sucrose. Sucrose having half of the molecule (fructose) labeled with C[14] has thus been synthesized. This reaction may be written as follows:

$$\text{D-glucose-1-*fructoside} + \text{enzyme} \rightleftharpoons \text{glucose-enzyme} + \text{*fructose}$$
(sucrose)
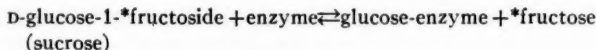
It should be noted that in both reactions, the enzyme acts as glucose donor and acceptor to its substrates, and can catalyze the exchange of an ester bond for a glycosidic linkage.

The transglucosidase function of sucrose phosphorylase was further indicated by the demonstration that the enzyme catalyzes an exchange of glucosidic bonds between two different disaccharides in the absence of glucose-1-phosphate and inorganic phosphate (38). Thus, the D-glucosido-L-sorboside, which was originally synthesized from glucose-1-phosphate and L-sorbose, was prepared by a reaction between sucrose and L-sorbose:

$$\text{D-glucose-1-fructoside} + \text{sorbose} \rightleftharpoons \text{D-glucose-1-sorboside} + \text{fructose}$$
(sucrose)
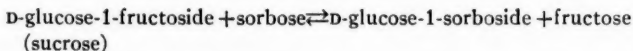
Similarly, sucrose was prepared by a reaction between the synthetic disaccharide, D-glucosido-xyloketoside and fructose as follows:

$$\text{D-glucose-1-xyloketoside} + \text{fructose} \rightleftharpoons \text{D-glucose-1-fructoside} + \text{xyloketose}$$
(sucrose)
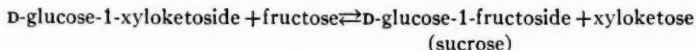
Both reactions are reversible and therefore provide a means of synthesizing any of the three disaccharides in either of two ways without the intervention of phosphate compounds.

Relation of sucrose phosphorylase to polysaccharide synthesizing enzymes.—

While sucrose phosphorylase of *P. saccharophila*, like the animal and plant phosphorylases, acts as a phosphorolytic enzyme, it is also related in its action to a group of bacterial enzymes known to catalyze polysaccharide syntheses which do not involve D-glucose-1-phosphate (40). These enzymes are capable of exchanging glycosidic linkages of disaccharides for those of polysaccharides and, like the sucrose phosphorylase, can therefore be considered as "transglycosidases."

A polysaccharide, dextran, is formed directly from sucrose by an enzyme of *Leuconostoc mesenteroids* (41). The reaction involves the substitution of a 1,6-glucosidic linkage for the glucose-fructose bond and can be represented by the following equation:

$$(n)C_{12}O_{22}O_{11} \rightarrow (C_6H_{10}O_5)_n + (n)C_6H_{12}O_6$$
$$\text{(sucrose)} \qquad \text{(dextran)} \quad \text{(fructose)}$$
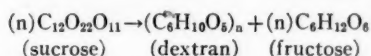
No evidence has been obtained for the reversibility of this reaction. When the enzyme is allowed to act on sucrose until no further chemical change occurs, the final sucrose concentration is very low. When reversibility was tested by incubating fructose and dextran with the enzyme, no sucrose could be detected in the reaction medium.

A similar exchange of glycosidic linkages is involved in the formation of a starch-like polysaccharide from maltose by an enzyme amylomaltase from *E. coli* (42). The reaction may be written as follows:

$$(n)C_{12}H_{22}O_{11} \rightleftarrows \quad (C_6H_{10}O_5)_n \qquad (n)C_6H_{12}O_6$$
$$\text{(maltose)} \quad \text{(polysaccharide)} \quad \text{(glucose)}$$
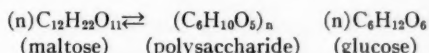
The same organism contains a phosphorylase which produces a similar polysaccharide from glucose-1-phosphate. The properties of the polysaccharide formed by amylomaltase depend upon the concentration of glucose in the reaction mixture. When glucose is continuously removed during the reaction, a product is obtained that stains deep blue with iodine, indicating that it consists at least partially of a polysaccharide similar to amylose. When glucose is allowed to accumulate during the decomposition of maltose, the product consists of reducing dextrins containing on the average from 4 to 6 glucose units. The influence of glucose on the molecular size of the polysaccharide can be interpreted in terms of the reversibility of the above reaction. By removing the glucose, the reaction would be expected to go to completion, whereas in the presence of glucose, the reverse reaction would lead to the partial depolymerization of the polysaccharide.

A number of species of bacteria belonging to different genera are capable of forming a fructose polymer, levan, from sucrose or raffinose (43). In this reaction a 2,6-linkage is substituted for the glycosidic bond of fructose, forming the levan:

$$(n)C_{12}O_{22}O_{11} \rightarrow (C_6H_{10}O_5)_n + (n)C_6H_{12}O_6$$
$$\text{(sucrose)} \qquad \text{(levan)} \qquad \text{(glucose)}$$
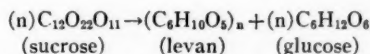
The reversibility of this reaction cannot be shown directly, probably, be-

cause the low molecular concentration of the levan displaces the equilibrium to the right. However, indirect evidence for enzymatic conversion of levan and glucose to sucrose has been obtained by the addition of invertase to the reaction mixture in order to displace the equilibrium to the left by decomposing sucrose as rapidly as it is formed. It was observed that the levan is broken down more rapidly in the presence of invertase and glucose than with either alone.

Hehre (44) recently obtained from *Neisseria perflava* an enzyme, amylo-sucrose, which has the ability to convert sucrose to an amylopectin-like poly-saccharide, presumably according to the following reaction:

$$(n)C_{12}H_{22}O_{11} \rightarrow \quad (C_6H_{10}O_5)_n \quad + (n)C_6H_{12}O_6$$
$$\text{(sucrose)} \qquad \text{(polysaccharide)} \quad \text{(fructose)}$$
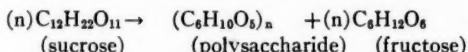
The reaction involves the substitution of a 1,4-glucosidic linkage for the 1,2-linkage in sucrose, without the mediation of glucose-1-phosphate.

The significant conclusion that can be drawn from these studies is that complex saccharides are never formed from unsubstituted monosaccharides. The formation of a glycosidic bond requires energy, and this must be supplied either in the form of a pre-existing glycosidic bond or as a C-O-P linkage such as is present in glucose-1-phosphate. This compound and its analogues may be the ultimate precursors of all glycosidic bonds since these phosphory-lated compounds lie on the only known path between simple sugars and complex saccharides.

There is good reason to believe that anhydro sugar units can be utilized for the synthesis of new glycosidic bonds only when they are present in the substrate in the form of glycosides. Since each half of the molecule in nonreducing disaccharides fulfills this condition, both sugar moieties can furnish units for polysaccharide synthesis. Sucrose, for example, can be converted both into dextrans and levans.

The formation of nonreducing disaccharides from glucose-1-phosphate (38) provides a general mechanism for raising free sugars to an energy level at which they can be used for the synthesis of other complex carbohydrates. Thus in the formation of sucrose from glucose-1-phosphate and fructose, the energy of the ester bond is used to transform fructose to a fructoside that in turn can serve as a substrate for polysaccharide (levan) synthesis. It is quite possible that nonreducing disaccharides may play an important rôle in the synthesis of plant polysaccharides, such as inulin and other fructosans, pentosans, and pectins.

Synthesis of starch.—The phosphorolytic degradation of starch (3) and related polysaccharides (45) involves the removal of successive glucose units at the nonreducing end of the chain as the result of a reaction with inorganic phosphate. Unlike the hydrolysis of starch by amylase, the phosphorolytic reaction is reversible, which means that starch can be synthesized from glucose-1-phosphate. The reversibility of the reaction,

$$\text{starch} + (n)HPO_4^{--} \rightleftarrows (n)\text{glucose-1-phosphate},$$
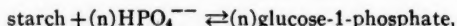
is due to the fact that the energy of the C-O-P linkages of glucose-1-phosphate (4,800 calories) is approximately the same as that of the glucosidic linkages of the polysaccharide, indicating that the $\Delta F°$ for the phosphorolytic reaction must be very small (46). The equilibrium of the phosphorolysis reaction is not affected by the concentration of the polysaccharide provided a certain minimum concentration is exceeded (47). This can be explained by the fact that the polysaccharides are very large molecules and are degraded by progressive removal of terminal glucose units without altering the number of reacting groups. However, the equilibrium is markedly affected by the change of the relative concentrations of inorganic phosphate and glucose-1-phosphate. It therefore follows that any process in the living cell that causes a decrease in the ratio of inorganic phosphate to glucose-1-phosphate favors polysaccharide synthesis.

Because glucose-1-phosphate is a stronger acid than orthophosphate, the equilibrium also depends on the hydrogen ion concentration; thus when the pH value of the system is varied from 5.0 to 7.0, the ratio of inorganic phosphate to ester-phosphate decreases from 10.8 to 3.1 (48).

It has been observed that when highly purified potato phosphorylase and synthetic glucose-1-phosphate are used, formation of starch does not take place, unless a small amount of starch, glycogen, or dextrin is added as a priming agent. The highly branched polysaccharides, amylopectin and glycogen, are most effective as primers for the reaction. It is assumed that a primer is required because the enzyme is unable to cause a condensation of glucose-1-phosphate units, but acts as a medium for transferring glucose units from glucose-1-phosphate to the end of an already existing polysaccharide chain, as shown by the following reaction (45):

$$(C_6H_{10}O_5)_x \quad + \quad C_6H_{11}O_6OPO_3{}^{--} \rightarrow (C_6H_{10}O_5)_{x+1} + HPO_4{}^{--}$$
Activating polysaccharide   glucose-1-phosphate
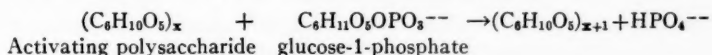
Apparently, when the chains reach a certain length they separate from the primer, producing long unbranched molecules.

Potato phosphorylase does not produce a typical starch *in vitro;* neither does the muscle phosphorylase synthesize a polysaccharide *in vitro* similar to animal glycogen. In both cases the polysaccharides synthesized *in vitro* are similar to the amylose fraction of potato starch (49). Cori & Cori (50) regard the synthesis of the branched glycogen from glucose-1-phosphate as the result of collaboration of two enzymes, one of which produces chains having 1,4-glucosidic linkages, while the other presumably forms 1,6-glucosidic linkages at the point of branching.

The possible existence of two such enzymes was demonstrated by Haworth, Bourne & Peat (51) who isolated from the potato a 1,6-linking enzyme which presumably, in conjunction with potato phosphorylase, catalyzes the synthesis of amylopectin from glucose-1-phosphate. The 1,6-linking enzyme is referred to as the "Q-enzyme," while the potato phosphorylase as purified by Hanes' method (3) is called "P-enzyme." A combination of P- and Q-enzymes acting upon glucose-1-phosphate yields a branched polysaccharide

resembling amylopectin. In this scheme of synthesis the Q-enzyme functions as a non-phosphorolytic enzyme.

Evidence indicating the existence of two types of enzymes participating in the synthesis of branched polysaccharides is also presented by Bernfeld & Meutémédian (52). These workers claim to have isolated from potato extract an enzyme, isophosphorylase, which, in the presence of inorganic phosphate will convert amylose to a branched polysaccharide that is hydrolyzed with β-amylase to 65 per cent maltose. No conversion takes place in the absence of phosphate. It should be noted that Haworth, Peat & Bourne on the one hand, and Bernfeld & Meutémédian on the other, claim fundamentally different mechanisms for the synthesis of branched polysaccharide in the same plant. It appears that while there is some experimental data to indicate that the existence of two separate enzymes is involved in the synthesis of branched amylopectin, the mechanism of branching is still an open question.

Formation of pentoses.—It has often been assumed that pentoses are formed through decarboxylations of uronic acid residues in polyuronides, which had been derived by oxidation of the primary alcohol groups in hexosans. Thus, for example, D-xylose in the polysaccharide xylan which exists in close association with cellulose may theoretically be derived from D-glucose of the latter polysaccharide. Similarly, L-arabinose in the polysaccharide araban may have its origin in the D-galactose of its polymer galactan.

However, there is no experimental evidence to support the idea that pentoses are formed by this mechanism in plant tissues. No enzyme has ever been found in plants which will decarboxylate uronic acids with the formation of the related pentose. In fact, there is evidence that appears to be inconsistent with the "decarboxylation theory." Hirst (53), in examining the structure of two closely associated polysaccharides, a galactan and an araban, found that the galactose residues of the former polysaccharide had the pyranose configuration, whereas the arabinose residues of the latter possessed the furanose configuration. The arbinofuranose units could not have been derived directly from galactose by oxidation and subsequent decarboxylation, since such a transformation should yield arabinopyranose units.

The work of Meyerhof, Lohmann & Schuster (54) with animal aldolase and that of Stumpf (24, 25, 26) with plant aldolase suggest a possible mechanism for the formation of pentoses in living cells. Meyerhof demonstrated that aldolase will catalyze the reversible condensation of a number of aldehydes with dihydroxyacetone phosphate. For example, with acetaldehyde, 5-desoxy-ketopentose-1-phosphate is formed according to the following reaction:

$$CH_2OHCOCH_2OPO_3H_2 + CH_3CHO \rightleftarrows CH_3CHOHCHOHCOCH_2OPO_3H_2$$

With glycolic aldehyde, a phosphorylated ketopentose is formed:

$$CH_2OHCOCH_2OPO_3H_2 + CH_2OHCHO \rightleftarrows CH_2OHCHOHCHOHCOCH_2OPO_3H_2$$

Similarly, in the reaction with a tricarbon aldehyde, such as D-glyceraldehyde, D-fructose-1-phosphate is obtained.

Because of the reversible nature of the reaction catalyzed by this enzyme, Bell (55) suggested this mechanism as a possible mode for pentose as well as hexose sugar synthesis. The idea of enzymatic synthesis of larger carbon chains out of small fragments is supported by the work of Stumpf (24, 25) who demonstrated that the enzyme, aldolase, is practically of universal occurrence in plants. At present this seems to afford a more reasonable hypothesis for the origin of pentoses than the theory of shortening the carbon chain of a hexuronic acid by loss of carbon dioxide from the carboxyl group of the sixth position.

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GROWTH REGULATING SUBSTANCES IN HORTICULTURE

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Introduction

In this review an attempt is made to present some of the new ideas, theories and results that have developed from recent research on plant growth regulating substances. The period reviewed covers mainly the last five years. Since it is not possible within the limits of this paper to refer to all work on growth regulators accomplished during this period, some reports have been selected that illustrate valuable new types of responses and their physiological aspects are discussed.

One of the most important practical uses of growth regulating chemicals involves their herbicidal effects on plants. This subject is not reviewed here since it is covered elsewhere in this journal.

Absorption and Translocation of Growth Substances

The type and magnitude of responses obtained with growth regulating chemicals depend upon the amount of the compound absorbed by the plant, the amount translocated within the plant, and the ability of the plant to respond to the stimulus of the particular chemical that is used. Most synthetic growth regulators are readily absorbed by any living surface cells of the plant. Some parts, such as the roots, may absorb these chemicals somewhat more readily than do others, such as a mature portion of a stem. Light and temperature are factors that affect the rate of absorption of certain growth substances. According to Rice (94), absence of light and decrease in temperature reduced absorption of 2, 4-dichlorophenoxyacetic acid (2, 4-D) in his experiments with bean plants.

Wetting agents and some hygroscopic substances have a marked effect upon the rate of absorption of such a growth regulating substance as 2, 4-D. Adjuvants, such as Carbowax, Tween-20, and various other detergents, act as solvents and also as spreading agents, thus holding the growth substances in a finely divided state and in close contact with the surface of the plant [Ennis & Boyd (31), Hitchcock & Zimmerman (50), and Mitchell & Hamner (78)].

Translocation of growth regulators absorbed by roots apparently occurs mainly in the water-conducting tissues [Hitchcock & Zimmerman (47, 50) and Mitchell & Brown (77)]. When absorbed by leaves, the chemicals are apparently translocated to the stem and other parts of the plant in a manner similar to that in which carbohydrates are translocated. Several investigators have shown that growth regulators of the phenoxy type are not

readily translocated from leaves to stems of plants under conditions unfavorable for the translocation of photosynthate (58, 77, 94).

Mitchell, Wirwille, & Weil (84) reported that certain nicotinium compounds, such as 2,4-dichlorobenzylnicotinium chloride, caused growth effects when applied to the stems of bean plants, but these compounds were ineffective when applied to leaves or cotyledons of the plants. Thus there is reason to believe that the mechanism by which plants translocate different growth substances may vary, but these processes are not yet understood.

VEGETATIVE PROPAGATION

The early discovery that growth regulating chemicals could be used as an aid in vegetative propagation resulted in numerous papers on the subject during the period 1938 to 1942. Results of this work, dealing mainly with practical problems about propagation, have been summarized by Mitchell & Rice (80) and by Thimann & Behnke (112). From 1941 to 1945, during the period of national emergency, research in this field was directed mainly toward the propagation of critical plants. Since then the rooting response of cuttings has received relatively little attention. Although growth regulating chemicals are now widely used in a practical way by nurserymen, information regarding the physiological and ecological aspects of this response is limited.

The theory that a natural root-inducing factor moves from the leaves to the basal part of the cuttings and causes root initiation has long been entertained. The basis of this theory rests in the fact that leafless cuttings often root less readily than do leaf-bearing ones [Cooper (18, 20)]. Recent experiments support this theory as far as a root-inducing factor is concerned, but the nature of this factor is yet unknown. Proof that some leaves produce a root factor has been clearly demonstrated by Gregory & Van Overbeek (38), using red and white varieties of *Hybiscus*. White *Hybiscus* leaves apparently lack a root-inducing factor and the cuttings themselves also lack a second factor which can be supplied in the form of indolebutyric acid. Thus cuttings of the white *Hybiscus* lost their leaves and failed to root; but when a stem bearing the persistent leaves of the red variety was grafted onto a cutting of the white variety, then an application of indolebutyric acid induced rooting. This experiment does not indicate the nature of the root-inducing factor supplied by the leaves. It may have been nutritional in character, or a hormone which functioned in the presence of the growth regulator, or a combination of both of these factors.

From a nutritional standpoint, carbohydrates and nitrogenous materials are mobilized in the basal ends of untreated cuttings, the region where root initiation occurs (81, 110). The application of growth regulating substances speeds this process of mobilization which apparently includes the accumulation of minerals and all other factors required in the production of cells (12). There is no proof, however, that the effect of growth regulators is directly on this process of mobilization. The synthetic stimulus apparently affects an early stage in the process of cell proliferation, since such changes

as increase in cell size, nuclear activity, and increased density of cytoplasm are accelerated by indolebutyric acid and naphthaleneacetic acid [Kraus *et al.* (40, 57)].

Many growth regulating substances have been tested for root-inducing activity. Among the more promising new ones are some phenoxy compounds, including 2, 4-dichlorophenoxypropionic acid, 2, 4, 5-trichlorophenoxypropionic acid, and 2, 4-dibromophenoxypropionic acid. The better known root-inducing substances, such as indolebutyric acid, naphthaleneacetic acid, and naphthaleneacetamide, still rank high from the standpoint of their general usefulness in vegetative propagation (24, 49, 90).

Some new methods of applying root-inducing chemicals have been developed. The treatments have been effectively applied by momentarily dipping the basal ends of the cuttings in a concentrated alcoholic solution of the growth regulator [Cooper (17) and Hitchcock & Zimmerman (48)]. Treatments applied to the tops of cuttings after they are planted are also effective [Hildreth & Mitchell (46)]. Root initiation has also been stimulated by spraying the attached branches of a plant with the growth regulator before the stems are detached and made into cuttings (108).

McClellan & Stuart (70) and Hartman (45) found that some kinds of cuttings, including those from gladiolus corms and narcissus bulbs are relatively susceptible to attack by fungi. Treatment of the cuttings with a combination of fungicide and growth regulating substance (indolebutyric acid or naphthaleneacetic acid) has experimentally stimulated root initiation and reduced the amount of decay caused by the fungi.

Light quality and duration of photoperiod affect the rooting of cuttings. Stoutemyer (106, 107) reports that light from artificial sources has been used successfully in rooting cuttings treated with growth regulating chemicals. He found that the length of photoperiod and the quality of light sometimes influence the manner in which certain cuttings respond to growth regulating chemicals. In general, the orange-red end of the spectrum was found to be most favorable for rooting purposes. That the duration of the photoperiod may influence root initiation has been demonstrated with Chinotto orange cuttings. Untreated, these rooted most prolifically when kept in continuous light. Treated with growth regulating chemicals, they developed more roots when exposed to a 16-hr. photoperiod than when subjected to uninterrupted illumination (106, 107). In contrast, other kinds of cuttings respond favorably to growth regulators irrespective of photoperiod.

It has been a general observation that some kinds of cuttings can be overstimulated in root production, thereby utilizing a high percentage of their food reserves. This factor in combination with direct bud-inhibiting effects of the root-promoting substance may account for reduced top growth that is sometimes found with growth-regulator-treated cuttings.

A study of the physiology of root initiation as influenced by growth substances is complicated by the fact that wide differences exist in the plant material tested. Research in this field has mainly been directed toward studying comparative responses from the standpoint of practical applica-

tions. There is need for greater emphasis on research dealing with the physiological aspects of the effect of growth regulating chemicals on root initiation.

FRUIT GROWTH AND MATURATION

Growth regulating substances have, under certain conditions, a prolonged effect on the growth of fruits. Howlett and others (52, 87) have found that application of indolebutyric acid to the flowers of a tomato plant inhibits subsequent seed development and, at the same time, stimulates growth of the fruit, an effect that persists with the result that the final size of the fruit often exceeds that of the untreated one. This prolonged growth effect is possibly secondary in nature or it may be due to a gradual absorption of the minute amount of the chemical required to cause parthenocarpy. There is evidence from tests with radioactive tagged compounds that the growth regulator, once absorbed, may be irreversibly combined with some constituent of the fruit [Mitchel *et al.* (85)]. From this standpoint, it appears that a continual supply of the growth substance would be required for a response to continue during the entire development of the fruit.

Wittwer & Murneek (124) obtained a prolonged stimulation of fruit growth after applying 4-chlorophenoxyacetic acid and other phenoxy compounds in concentrations of a few parts per million in water to blossoms of snap bean plants grown under relatively dry field conditions. In these experiments maturation of the fruits was hastened and their final size was increased. Application of growth substances to partially developed fruits has also produced a growth effect which persists during their subsequent development. Marth & Havis (64) have increased the growth rate of peach fruits, for instance, by spraying them in the preripe stage with 2, 4, 5-trichlorophenoxyacetic acid (2,4,5-T). The chemical increased growth of the seed and pericarp, and this increased rate of development persisted, with the result that the fruits matured and ripened 5 to 30 days earlier than did untreated ones. Blondeaux & Crane (10) found that the same compound had a similar stimulating effect on the rate of maturation of fig fruits (Lob Ingir). In contrast, however, maturation of snap beans was retarded by 2,4,5-T, 4-chlorophenoxyacetic acid, and other growth regulating substances when applied in relatively high concentrations of 200 to 400 p.p.m. to the partially developed fruits [Mitchell (74)].

At present we have no knowledge as to how growth regulating substances affect the various metabolic processes of the fruits of peach, fig, and bean so as to stimulate their growth and hasten their ripening. With respect to retarding the maturation of bean fruits through the use of relatively large amounts of the chemicals, the response is associated with chemical changes which first cause the fruits to absorb more water than they normally contain (74). In addition, the treated fruits were apparently changed chemically so that they retained the water against forces of evaporation more tenaciously than did untreated ones. The possibility that growth regulating substances may alter some proteinaceous constituents of cells so as to increase their affinity for water is suggested as a possible factor in accounting for the

increased water-retaining capacity observed. Evidence has not yet been obtained, however, to support this theory.

Growth-regulating substances can alter the rate of ripening of attached fruits, such as apples, when these are sprayed with very minute amounts of the chemical (37, 42, 99, 100). Batjer & Moon (5) found that a spray mixture containing as little as 0.001 per cent of naphthaleneacetic acid hastened the rate of ripening of Close, Williams, and Duchess varieties of apples by a few days, while stronger applications of 2, 4, 5-T hastened ripening of the Rome variety of apple by a month [Marth, Harley & Havis (63)].

It is not essential, however, that fruits be attached to the plant in order to accelerate their rate of ripening with growth regulators. Mitchell & Marth (79) increased the ripening rate of detached apples, peaches, and pears by the application of 2, 4-D to the fruits that were picked and treated before they were fully ripe. Chemical changes that are accelerated by treating detached fruits with growth regulators are apparently similar to those that are normally involved in the ripening of untreated fruits. When the growth regulator treatment is properly used, the quality of the artificially ripened fruits is equal to that of those that ripen naturally.

Study of the effects of growth-regulating substances on the ripening rate of detached bananas has brought to light a possible relationship between substances emanating from fruits and chemically-induced ripening. Ripening of detached banana fruits was hastened by applications of 2,4-D. This response occurred when the fruits were well ventilated following treatment. In subsequent work (65) treated fruits that were confined within an air-tight chamber failed to respond to the synthetic growth regulator, but they responded readily to artificially added ethylene and to ethylene produced by the fruits themselves. The results suggest the possibility that a volatile substance produced by the enclosed fruit inactivated the ripening effect of the 2, 4-D but not that of ethylene.

Ripening processes of some fruits, such as those of citrus, have not as yet been accelerated by the use of synthetic growth substances, but the rate at which they color is readily hastened when the natural growth regulator (ethylene) is supplied artificially (123). Hansen (42) reported that Bartlett pears are sensitive to combined treatments of ethylene and 2,4-D. Banana fruits, on the other hand, were found to be insensitive to ethylene after they had been treated with the acid, for they ripened at the same rate as when the acid was used alone (65).

Thus, the maturation of various kinds of fruits can be accelerated, while that of others can be retarded through the use of growth regulating substances. Enzyme activity is directly or indirectly involved in these responses, but the exact nature of these chemical effects is not known.

EFFECT OF GROWTH-REGULATING SUBSTANCE ON FLOWER ABSCISSION AND FRUIT SET

In some plants the abscission of flowers is readily retarded by applications of certain growth-regulating substances (53, 56, 129). Other plants in the same stage of development respond in an opposite way and abscission

may be accelerated by the same growth regulator applied over a wide range of concentrations (6, 22, 88, 101, 102). Penetration and transport [Mitchell & Brown (77), Wester & Marth (119)] of the growth regulator appear to be factors involved in preventing abscission, at least with certain plants. For instance, Clore (16), and Wester & Marth (119) found that field and greenhouse spraying of flowers of bush lima beans was relatively ineffective in improving pod set. However, in later work (120) the chemicals were applied as a relatively concentrated lanolin paste and the flower stalks were punctured slightly. Abscission was retarded and pod set was greatly improved by this method.

The abscission of flowers of greenhouse tomatoes is readily prevented by treating them with growth regulators according to Howlett (52, 53) and Zimmerman & Hitchcock (129). However, Mullison (87) reports that the response of different varieties is not always the same. Poorly filled fruits that sometimes result from application of growth regulator to certain varieties seems to be due to unequal stimulation in the development of carpel walls and placental tissues [Hamner, Schomer, & Marth (41)]. Recently, it has been demonstrated by Randhawa & Thompson (93) that uptake of the growth regulator through the roots of tomato plants results in greater development of the placenta and more complete fruit filling.

Growth-regulating substances have shown some promise as a supplementary treatment in promoting cross-pollination [Emsweller & Stuart (30), Wester & Marth (119), Whitaker & Pryor (121)], but at present there is no reliable method of selecting compounds on the basis of chemical structure or of activity on one kind or variety of plant, for use on another. The development of seeds may be sharply reduced by growth regulator treatment [Hardenburg (43), Wittwer & Murneek (124), Zimmerman & Hitchcock (129)], and studies are needed to determine the most desirable time of application of pollen as well as growth regulator when seed production is the objective. Another consideration in using growth regulators for this purpose is a possible carry-over effect of the chemical into the progeny [Pridham (91)], resulting in malformed seedlings. With certain cereal and grass crops this does not appear to be an important consideration [Marth & Toole (67, 68)].

Early work by Roderiguez (95) indicated that pineapple plants can be induced to flower by treatment with ethylene gas. Later Clark & Kerns (15), working in Hawaii, found that flowering of pineapple could be either hastened or retarded by treatment with naphthaleneacetic acid, depending upon the concentration used. About the same time Cooper (19) in Florida reported that pineapple could be induced to flower by growth-regulator applications made to the plant during October, but was apparently not responsive when treated in July. More recently Van Overbeek (115) reported that some varieties of pineapple could be induced to flower at any season of the year in Puerto Rico by treatment with 2,4-D as well as with naphthaleneacetic acid.

Zimmerman & Hitchcock (128, 130) treated tomato plants with 2, 3, 5-

triiodobenzoic acid (TIB) and induced flowers to grow from axillary buds where leafy shoots normally appear. Other workers have also obtained this response with tomato [Waard & Roodenburg (116)]. Galston (35) concluded from work with soybeans that TIB is not a florigenic substance since it did not cause vegetative soybeans to flower, but he did obtain an increase in the number of flowers that developed per plant. More evidence is needed to determine whether growth regulating chemicals affect the initiation of flower primordia in a variety of plants or merely influence the development of primordia already present at the time of treatment.

Effect of Growth-Regulating Substances in Retarding Fruit Drop

The experimental evidence is meager as to why application of growth-regulating chemicals may either hasten or retard abscission of leaves, flowers, and fruits (1, 4, 7, 9, 36, 44, 101, 122, 129). Anatomical changes preceding abscission of a given plant part may be quite different, from one time to another, depending on its stage of development (72).

After five years of experimentation with apple, McCown (72) has concluded that the shedding of its flowers and young fruits is preceded by the formation, through cell division, of a definite thin-walled abscission layer across the entire stem (pedicel) as previously described by Eames & Mac-Daniels (25). Dissolution of the middle lamellae, presumably by enzyme action, precedes abscission. Application of 10 p.p.m. of naphthaleneacetic acid at this early stage of development hastens abscission [Southwick & Weeks (101)].

In contrast, the harvest drop of mature fruits is not preceded by initiation of new cells but involves dissolution changes in the walls and lamellae of old, lignified cells. Harvest spraying with 10 p.p.m. of naphthaleneacetic acid will retard abscission at this time (4, 8, 62, 114).

Some varieties of apple show marked differences in the intensity of response to spray applications of naphthaleneacetic acid in retarding drop of fruits (3). Differences in time of fruit maturation, moisture supply, or other factors may account for some of the observed variation (3, 51). Applications of 2, 4-D have proved effective on two late-maturing varieties (Stayman Winesap and Winesap), although this compound is ineffective on many other late maturing varieties, which also often fail to respond well to naphthaleneacetic acid treatment. Moon & co-workers (86) have found that spray applications of 2, 4-D may have a hold-over effect from one harvest season to the next. This was a remarkable response since only 10 p.p.m. spray concentration of the chemical was originally applied.

Penetration of the chemical into the tree may be an important factor in some cases. With the McIntosh variety, Edgerton & Hoffman (26) report that external spray applications of naphthaleneacetic acid and 2, 4-D were without effect in preventing or reducing premature drop, but injections of the chemicals into the tree retarded fruit abscission at some distance from the point of injection. Batjer & Thompson (8) found that when naphthalene-

acetic acid was applied as an external spray to a responsive variety the stimulus moved only short distances and the effect on the fruit was primarily from absorption by leaves adjacent to the fruit rather than through fruit surfaces. This was determined by applying the growth regulator solution to fruit stems and cluster bases with a camel-hair brush in some treatments, while in others the spur leaves adjacent to the fruit were sprayed with a hand atomizer. A much reduced effect of the chemical in preventing fruit drop was obtained by treating the stems only, in comparison with the leaf treatments. Greatest retardation in drop, however, occurred when the entire spur and leaves were sprayed. These workers found that some of the growth regulator stimulus moved from one spur to another when in close proximity (compound spurs); but when situated at a distance of six inches or more there was no effect of a treated spur on an untreated one. It is of interest that in Michigan some varieties respond to 2-methyl-4-chlorophenoxyacetic acid, but show little or no response to the closely related 2, 4-dichlorophenoxyacetic acid [Mitchell et al. (83)].

A marked selectivity for certain compounds with respect to preventing drop of mature fruit is shown by a number of different plants. The drop of citrus fruits (orange, grapefruit, lemon) was not retarded by application of naphthaleneacetic acid, but recently Stewart (105) found that application of 2,4-D causes these fruits to hang tenaciously. On the other hand, results reported by Crane and co-workers (10, 21) with another phenoxy compound, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), on Lob Ingir (Calimyrna) figs indicate that the chemical not only retards mature fruit abscission but also greatly hastens ripening, while similar applications of 2,4-D failed to have an effect on either abscission or ripening.

It would seem that greater advances could be made in using growth regulating substances to control fruit abscission if more data were at hand on penetration and movement of the various chemicals after application, as well as on the nature of the internal physiological processes that are under way at the time of treatment and which must be influenced to bring about the response desired.

Effect of Growth-Regulating Substances on Dormancy

Considerable experimental evidence has accumulated in support of the theory that a bud-inhibiting substance is formed in plants. Pfeffer (89) as early as 1903 suggested that since dormant lateral buds contain adequate food reserves for growth, their suppression is caused by some other factor than food alone. Appleman (2) found that if potato tubers were left intact their apical bud showed greatest development, whereas upon division or by cortical incision between buds they were all induced to grow. He concluded that the bud inhibitor moved in the cortex. Snow (98) was able to demonstrate with bean plants that a definite bud-inhibiting substance was formed in plants, and later Thimann & Skoog (113) were able to induce bud-inhibiting effects with synthetic growth regulators as well as with extracts of natu-

rally occurring growth substances. Guthrie (39) found that all the buds of potato tubers could be inhibited by solutions and vapors of synthetic growth regulators, and in a manner very similar to inhibition by apical buds.

Elmer (29) states that the gaseous emanations from ripening apples cause a retardation in the bud development of unsprouted potato tubers that had completed their rest period, similar to that obtained by treating the tubers with synthetic growth regulators. He concluded that the growth-inhibiting gas produced by ripening apples and pears is probably ethylene. However, in this work no growth-inhibiting gases that would affect potato buds were obtained from banana fruits, although ripening banana fruits produce an abundance of ethylene which has a drastic epinastic effect on the growth of young tomato plants [Marth & Mitchell (65)]. It seems possible to conclude that ripening fruits produce growth regulators of the ethylene type as well as others that may differ in their action on plants.

The mechanism by which synthetic growth regulators prolong dormancy in stored potato (23, 28, 54) and other crops (54, 60, 61, 103, 117) is not yet understood. Mechanical drying is not a recommended practice but is a practical method sometimes resorted to for the purpose of prolonging dormancy in plants. Since growth-regulating substances have a pronounced effect on movement of water and other materials from one plant part to another and on their accumulation in certain tissues, it is conceivable that such shifting may result in physiological dryness in the region of the vegetative buds and temporarily delay bud growth. Ellison & Smith (28) and Fults & Schaal (34) have shown that growth-regulating substances can be applied to potato tops in the field and the stimulus will then be translocated down into the tubers, affecting the color, quality, and dormancy when they are later held in storage.

Plants may be quite selective in their sensitivity to growth regulators applied for the purpose of prolonging dormancy in storage. Potato tubers show no marked bud-inhibiting effects from applications of 4-chlorophen-oxy-acetic acid, 2,4-D, or 2,4,6-trichlorophenoxyacetic acid; however, the closely related phenoxy compound 2,4,5-T is very potent in its bud-inhibiting effects on potato tubers [Ennis et al. (32), (Marth & Schultz (66), and Wood & Ennis (125)]. The use of 2,4,5-T to prolong dormancy of stored table-stock potatoes has not been developed sufficiently to warrant recommending its use. However, this growth regulator shows promise of immediate usefulness in controlling the sprouting of potatoes on cull piles which constitute a source of infection of certain diseases and insects in the large producing areas (66).

Explanation of why there is a selective action among the phenoxy compounds as well as other growth regulators in bringing about a given plant response is yet to be sought. On the basis of work with radioactive tracers by Wood et al. (126) and Mitchell et al. (85), it may be assumed that the mere entrance of one growth regulator versus another is not always a critical consideration, but the chemical composition of the particular plant at the time of treatment has an important bearing on the growth effects obtainable.

In connection with studies on the effects of growth regulators on vegetative bud dormancy, rose bushes with relatively large amounts of carbohydrate reserve were much more responsive and less readily injured by growth-regulator treatment than those lacking in reserves [Marth (61)]. Undoubtedly factors other than food reserves are involved, since the movement of water [Brown (11)] and minerals [Brunstetter *et al.* (12)] is also drastically changed by growth-regulator treatment.

OTHER RESPONSES

Numerous new ways in which plants respond to growth regulators have been discovered during the last five years. Most of these have so far received little attention from the standpoint of the physiological effects concerned. Among these responses is the effect of growth substances on the quality of plants and plant products from the standpoint of palatability. This type of response has been noted particularly in using phenoxy compounds as herbicides. Cattle sometimes graze pasture plants sprayed with 2,4-D in preference to unsprayed ones, and they sometimes eat poisonous weeds that have been sprayed, while normally these plants would be avoided. The physiological basis for improved palatability may rest in part on the greater degree of succulence of treated plants [Brown (11)]. That treated plants sometimes contain relatively large amounts of sugars and unelaborated nitrogenous compounds may also be a factor. Mitchell *et al.* (82) and Luecke *et al.* (59) have reported that the vitamin content of some pasture plants may also be affected and there is evidence that palatability of the plant as a whole may be improved because minerals (12), carbohydrates, and nitrogenous compounds (75, 76, 96, 97) are sometimes mobilized in parts of the plant that normally are relatively nonpalatable. The problem of the effect of growth regulating substances on the palatability and nutritional value of plants or specific parts of the plants would seem to merit greater attention.

With respect to lower plants, the development of horticulturally important fungi and bacteria is not greatly affected by growth regulating chemicals. Stevenson & Mitchell (104) found that some fungi, such as *Penicillium* species, are able to tolerate amounts of 2, 4-D that are toxic to many higher plants. Wood & Ennis (125) have presented evidence that some soil-borne organisms readily invade plants treated with growth regulators, and Martin (69) states that some can utilize growth regulating chemicals such as 2, 4-D as a source of energy. Stuart and McClellan (71, 111) found that treatment of scales of narcissus bulbs with indolebutyric acid favored the development of a *Fusarium* that grew on the plant material; and the addition of this growth regulator to artificial media also favored growth of the fungus on the media. The effect of the growth regulator in the latter case was directly on the fungus. An indirect effect on the growth of fungi has also been noted, however, since plants treated with 2, 4-D are often attacked by fungi more readily than are untreated ones. This may be due either to a lowered resistance of the host or to physiological changes within the host which make it more suited to the nutritional requirements of the pathogen.

The compounds 4-chlorophenoxyacetic and naphthaleneacetic acids have been used to retard dropping of petals of Japanese flowering cherry and of floral bracts of dogwood [Wester & Marth (118)]. These chemicals were ineffective in retarding the petal drop of many other ornamental species. In general, the abscission of petals differs from that of fruits. Abscission of apple and pear fruits can usually be retarded through the use of growth regulating substances. Kessler & Allison (55) found that abscission of the receptacle and calyx on lemon fruits could be retarded by treating the fruits with 2, 4-D. This response improved the storage quality of the lemons since they were less susceptible to attack by mold when stored with the "button" attached than when it had abscised.

Fults & Schaal (34) and Ellis (27) report that although most potato varieties are relatively insensitive to 2, 4-D, application of the chemical to the tops of the red variety Bliss Triumph caused the tubers to develop skins of an unusually deep red color. Treated tubers showed somewhat better storage quality than the untreated ones. Prince & Blood (92) have found that the cooking quality was improved. The chemical responses that account for these effects are unknown.

Acceleration of cell elongation is a well known response to such growth regulating substances as indoleacetic acid and 2, 4-D. Recently several nicotinium compounds were found to have an opposite effect of inhibiting internodal elongation of bean seedlings. When stems of the plants were treated with benzyl 2,4-dichloronicotinium chloride, for instance, elongation of the treated internodes and those that developed subsequently was greatly reduced, which indicated that the growth regulator was translocated from the point of application to the terminal buds where it checked elongation of the stems [Mitchell *et al.* (84)].

Leaves of many kinds of plants are sensitive to growth regulating substances. Leaf abscission has been delayed through the use of these chemicals, naphthaleneacetic acid being effective when sprayed on holly leaves [Milbrath & Hartman (73) and Worley & Grogan (127)]. In a different type of leaf response, growth regulators have been used to retard yellowing and delay abscission of the outer leaves of stored cauliflower [Carolus *et al.* (13)]. Indoleacetic acid has been used experimentally by Franklin (33) to cause epinasty in outer leaves of lettuce so as to produce less compact heads and to favor seed stalk development. Clark & Wittwer (14) reported that such widely different substances as 2, 4-D and TIB acid hastened elongation of the seed stalks of lettuce and celery plants. Still a different type of response has been reported by Prince & Blood (92), who found that seed-ball production of potatoes was increased with respect to size and number by spraying the flowering plants with 2, 4-D.

With respect to the growth of entire plants and the effect on fruit production, Stromme & Hamner (109) found that the rate of maturity of bean plants was retarded by application of sodium 2,4-dichlorophenoxyacetate at a concentration of 100 p.p.m. or less. At 1 or 10 p.p.m. the number of pods produced per plant was increased. The increased production was attributed

to an indirect effect involving stimulated axillary growth. This is an effect opposite to that repeatedly found in much of the earlier work. This is not a unique situation since in much of the experimental work with growth regulating substances diversity in type of response has been observed. The response obtained frequently depends upon (a) the kind or variety of plant used, (b) the part of the plant treated and its stage of development, (c) the amount of chemical absorbed, and (d) the kind of chemical applied. With so many factors involved, it is to be expected that a wide variety of responses and sometimes seemingly opposite effects will be obtained, and these will be difficult to interpret until we have gained a better understanding of the basic nature of plant responses to growth substances.

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HERBICIDES[1]

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The desirability of elimination or control of weeds in agricultural land has long been recognized and toxic chemicals have been employed for half a century as aids in eradication or suppression. Within the past few years however, owing to the discovery of markedly superior and economical types of herbicides, both the extent of usage and the variety of applications of chemical weed control have been extended greatly. Concomitantly, the scientific literature in this field has undergone rapid growth; several reviews dealing with various phases of the subject have appeared during recent years (2, 5, 19, 42, 63, 83, 129, 158, 195, 224, 232, 252, 302, 318, 324). In the present article an attempt has been made to describe briefly the current status of herbicides and also to emphasize certain aspects which have received relatively little attention elsewhere.

Herbicidal Agents

The number of chemicals which, in sufficiently high concentration, are capable of affecting plants adversely is virtually unlimited. At various times a large number have been advocated for herbicidal use and many have been offered for sale. Relatively few have withstood the test of time. Certain regional differences in adoption and utilization have arisen for reasons relating to cost or availability rather than to any particular property of the agent as an herbicide.

Inorganic compounds.—The first commercially developed herbicides were inorganic, but relatively few of these are now in use largely because of the high dosage rates required. A list of inorganic formulations has been published (121).

Sodium chlorate, which is probably the most widely used inorganic herbicide, is now employed principally for spot eradication of noxious weeds such as bindweed, Johnson grass, and Bermuda grass (104, 157, 262, 264). The sodium and magnesium salts appear to be more toxic than those of iron, calcium, or barium (239). The physical characteristics of the soil may be affected by heavy applications or continued use; alkaline soils of the Yakima Valley so treated showed a tendency toward puddling and caking on drying (149). Heavy soil applications may prevent re-establishment of vegetation for many months. Chlorates are strong oxidants and create a fire hazard in the presence of easily oxidizable material such as clothing or dry vegetation.

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[1] Most of the publications reviewed in this paper appeared in the years 1946 to 1949, inclusive.

[2] In the assembly of information and preparation of certain sections of this paper we were much aided by our colleagues R. M. Acker, J. W. Brown, G. E. Davis, W.B. Ennis, Jr., R. E. Hay, and A. S. Newman.

A number of arsenic compounds have been used for a variety of purposes. A recent application is the suppression of weeds in coconut palms (291). Lead arsenate has been suggested for control of crabgrass (319). Sodium arsenite may persist in soil for several years with continued effectiveness (80). To achieve both quick action and persistence a combination of sodium arsenite and arsenic trioxide has been recommended for mixtures of shallow and deep rooted plants (80). Arsenic trioxide has been used successfully as a preharvest potato vine killer without detrimental effect on the seed quality of the tubers (205).

Boron, although essential in minute amounts for plant growth, is highly toxic at somewhat greater concentrations. Field bindweed has beeen controlled with borax (157). This compound has been recommended for use only in nonagricultural areas. It disappears from soils fairly rapidly (147), being intermediate between chlorate and the arsenicals in this respect (80).

Calcium cyanamide is used both as a fertilizer and herbicide. Its herbicidal effect is due to toxic intermediate products of hydrolysis (182) which prevent weed seed germination. The toxicity is not long persistent and the compound is usually placed in the field a few weeks prior to planting. Calcium cyanamide has been used for controlling weeds in asparagus and cereals and in conjunction with uramon has been particularly effective in the preparation of tobacco seed beds (330). Sodium and potassium cyanates and ammonium thiocyanate, as well as sodium cyanide, have been used for the eradication of undesirable plants (38, 80, 96, 155, 203).

Ammonium sulfamate has been found in recent years to be a more suitable herbicide than sodium chlorate for certain troublesome weeds, including woody plants and particularly poison ivy (92, 106, 243, 323). Mixtures with chromate have successfully controlled quackgrass (21).

Oils.—Waste oils of various kinds (acid sludge, acid tar, Edeleanu extracts) and other by-products of oil refineries having little commercial value, have been employed as herbicides. Residues from coal and wood distillation have from time to time been utilized (85). Certain shale oil fractions have also been suggested (36). More recently, certain oils of substantially greater toxicity have been developed solely for herbicidal purposes. These include Shell Weed Killer No. 20, Avon Weed Killer, Standard (of California) No. 2, etc. (75, 85, 113). The heavy aromatic fractions appear useful for general killing of vegetation because they persist longer than the lighter oils (85).

Oils have been used to a large extent in a nonselective manner to kill all vegetation in such places as railroad rights-of-way, ditch banks and roadsides, fire strips, and in citrus orchards (214). For such practices diesel oil, kerosene, stove oil, crankcase oil, and miscellaneous crude by-products of oil refining have been employed (252).

With the discovery of differential tolerance of plant species to various oil fractions, work has been directed toward the development of selective oil herbicides. Stove oil and diesel oil have been used for selectively weeding guayule (32, 62). Notable success has been achieved in selectively weeding members of the Umbelliferae with stove oil (85, 284), the more expensive

Stoddard Solvent, and similar products (133, 158, 159, 186, 188, 189, 190, 234, 275, 283, 309, 327). Mineral spirits have been successfully used in controlling weeds in forest nursery seed beds (65). The content of aromatic substances and certain physical properties, such as the boiling range, are correlated with the toxicity of oils and consequently are of importance in selective uses (74, 85, 132, 152, 153, 154, 190, 197, 234, 283). There is need for greater uniformity and precision in describing the properties of particular oils used in herbicidal experiments and practices.

Phenols.—Sodium dinitro-*o*-cresylate (Sinox) was developed as an herbicide in France in 1933 and introduced into the United States a few years later (320). It rapidly found extensive use for controlling broad-leaved weeds in cereals (28, 93, 194, 247, 320) and for weed control in pea, corn, flax, garlic, and onion crops. Sinox has many advantages over the compounds previously used as selective herbicides in cereals; it is highly selective, noncorrosive, relatively nonpoisonous, and many investigators (51, 73) have reported actual stimulation of the treated crops. No hazard exists from accumulation of dinitro-*o*-cresol or its salts in the soil after repeated use because of the low application rates employed, rapid biological decomposition, and leaching. In the early period of its development the use of Sinox was somewhat limited because it was found to be ineffective when temperatures were below 50°C. at the time of application, or if the weather previous to spraying had been cold or excessively dry.

The toxicity of alkyl-substituted nitro- and chlorophenols increases with the size of the alkyl group and is influenced by number and position of other ring substituents (79). The substituted dinitrophenols are commonly designated as dinitros.

The most recent use for phenolic compounds is as fortifying agents for oils, the toxicity of which may be greatly increased in this way.

Phenoxyacetic acids (hormone-type herbicides).—The herbicides responsible for the renaissance of interest in chemical weed control in recent years and at present most widely utilized are substituted phenoxyacetic acids. The first representative of this group to be developed in this country was 2,4-dichlorophenoxyacetic acid (2,4-D), which is now manufactured in the United States to the extent of over 20 million pounds annually. This compound is included in many proprietary formulations as the sodium, ammonium or alkanolamine salt, or as an ester of one of the lower aliphatic alcohols.

Investigation of the herbicidal properties of the phenoxyacetates in England apparently antedated by several years the work done in the United States. The compound selected for development in Great Britain was 2-methyl-4-chlorophenoxyacetic acid (Methoxone) apparently because of the greater availability in England of chloro-cresol than chloro-phenol rather than because of the herbicidal superiority of Methoxone. Comparisons of 2,4-D and Methoxone have been reported by a number of investigators (9, 34, 53, 226, 297, 306).

Interest in 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as an herbicide has

increased in the last two or three years because of its specificity. Some species not responsive to 2,4-D are quite susceptible to 2,4,5,-T (66, 111, 274, 286). For control of woody species such as *Rubus*, mesquite, etc., 2,4,5-T is generally preferred (116, 125, 180, 211, 238, 240, 269, 274, 336).

Tests have been made of many other phenoxy compounds which possess physiological activity but which have not been produced on a commercial scale (127, 175, 225, 268, 285, 293, 297, 338). Some of these have activity of the same order as 2,4-D. The ready availability of 2,4-D and its high inhibitory properties have been responsible for concentration of effort on developing additional uses for this compound. Certain other phenoxy derivatives, although perhaps not so broadly effective, will probably be found particularly suitable in applications involving differential action.

As some of the esters of 2,4-D are volatile, damage to susceptible crops in fields adjacent to areas treated with them has been attributed to drift of the vapors. Volatility of esters has been blamed in some instances when injury more likely has been due to drift of droplets during spraying or failure to clean spray or dust rigs thoroughly after use with 2,4-D. Nevertheless it has been adequately demonstrated that vapors of 2,4-D esters can cause plant injury (174, 209, 226A, 278). Inasmuch as some esters of long chain aliphatic alcohols are less volatile than those of short chain alcohols, some producers are developing the former so that the advantages of esters may be retained while this undesirable characteristic is eliminated or minimized.

Carbamates.—O-Isopropyl-N-phenylcarbamate (IPC), although not yet proved as an effective herbicide, appears to possess interesting potentialities. Together with certain other urethanes it was found to be an inhibitor of growth of cereals (292, 294). The responses of various cereals and grasses have been carefully explored (10, 107, 108) and use of IPC for control of grassy weeds such as quackgrass has been proposed (57, 191, 223, 224, 326). Effective use of the compound in selectively killing wild oats in field-grown peas has recently been reported from Sweden (254). No obvious responses are produced in many dicotyledonous crop plants by this compound, although some seedlings are inhibited in various degrees (108). Differential use of IPC to control grassy weeds may be restricted for this reason.

Trichloroacetates.—Sodium and ammonium trichloroacetates appear to have promise as herbicides, nonselective in character, but particularly effective for control of grassy weeds, such as quackgrass, Johnson grass, and couch grass (27, 58, 59, 104).

Other organic compounds.—Some interest has been aroused by the possibility of employing phenyl mercuric acetate to prevent the germination and growth of crabgrass seedlings in turf, in grass seed beds, and for general weed control in gladiolus beds. Lawn grasses such as Kentucky bluegrass, Chewing's fescue, and Colonial bent are not permanently injured (94, 95, 128). Recently, sodium isopropyl xanthate has been under study as an herbicide, potato vine killer, and cotton defoliant (14, 30, 31). Salts of maleic hydrazide (1,2-dihydropyridazine-3,6-dione) at moderate rates have been demonstrated to induce a dormant condition in certain plants without sub-

sequent growth modifications (261) which might favor development of such compounds as selective herbicides. Prochlors, which are mixtures of chlorinated propane and propene, have been reported to control satisfactorily the growth of such weeds as Canada thistle, quackgrass, morning glory, and Russian knapweed, if injected into the soil to an appropriate depth (122). Chloropicrin has also been used effectively for the control of weeds (55, 90). The lachrymal properties of this compound make it difficult to handle and apply.

Combinations.—Mixtures of two or more active compounds may be of value in certain applications. As some weed species are more susceptible to one herbicide than another, suitable mixtures of herbicides might control many kinds of broadleaf weeds in a single application. Various mixtures are now offered for sale by herbicide manufacturers. Fortified oil emulsions, containing 2,4-D and pentachlorophenol, dinitro-amyl-phenol, or dinitro-butyl-phenol, form effective spray solutions for controlling mixed weed populations (82). Grassy weeds in Puerto Rico have been effectively treated with combination sprays containing pentachlorophenol and 2,4-D (207, 231). The incorporation of pentachlorophenol or sodium pentachlorophenate in 2,4-D solutions gave excellent weed control in sugar cane for as long as three months with no damage to the cane (140). A number of other fortified oil formulations have been tested (75, 76, 274).

Mixtures of phenoxy compounds have been tested with inconsistent results (9, 163). A 1:1 mixture of Methoxone and 2,4,5-T was more effective than either component for killing tomato and several weed species. It has been stated also that nonhormone herbicides can "activate" 2,4-D and that the converse also is true (163). Mixtures of 2,4-D and 2,4,5-T are now being recommended where coarse, woody vegetation is to be eradicated, as along highways, under electric power lines, and in similar areas (274). The value of such mixtures does not depend on synergism but is due to the diverse nature of the weed population being treated. Mixtures of 2,4-D and IPC are effective against both dicot and monocot seedlings (9) and have been suggested for weed control in set onions (191). A combination of phenyl mercuric acetate and 2,4-D is suitable for eliminating mixed populations of broadleaf weeds and crabgrass (94).

Uses of Herbicides

Herbicides are used in a variety of ways and for diverse purposes. Some compounds appropriately employed are satisfactory for a number of different uses; others, which are more limited in versatility, may nevertheless be equally valuable. The uses of herbicides may be classified as nonselective or selective, according as they are intended to eliminate all vegetation or only a portion of the flora. Selective uses in turn may be divided into (*a*) those directed against more or less established weeds in a growing crop, and (*b*) those designed for prevention of establishment of weed seedlings. These selective uses may also be designated as postemergence and pre-emergence, respectively, reference being made to the crop and not to the weeds.

Nonselective eradication.—Eradication of all vegetation and prevention of

reinfestation may be desirable in such applications as maintenance of railroad rights-of-way, fire breaks and along banks of irrigation ditches (80), control of aquatic plants in water ways and irrigation canals (71, 160, 164, 241), on tennis courts and around buildings, and for prevention of dissemination of weed seeds. Herbicides employed for such purposes have been referred to as soil sterilants. Several of the older materials (sodium chlorate, arsenicals, borax, sulfamate, carbon bisulfide) are highly effective. Although their usage probably has decreased with the advent of hormone-type herbicides they have characteristics which are advantageous in particular situations. Detailed information on usage, characteristics, and persistence of these compounds has been compiled (252) and recommendations for appropriate applications have been published (104, 262, 323).

Selective weed control in growing crops.—Of far greater agronomic and horticultural importance, however, are the differential and selective uses for herbicides that have been developed in recent years; some of these may confidently be expected to result in great changes in crop production practices (318). The essence of these selective treatments is that they are applied, almost invariably by spraying, when the crop is on the land, and have the effect of controlling competing weed species without influencing adversely the yield or quality of the crop.

Until quite recently, the spray volume rates employed were such that the entire foliage of the plant became wetted by the solution. Only since the introduction of 2,4-D has it been possible to kill plants with application rates as low as a few gallons per acre. In spray applications 2,4-D is now without doubt the most widely used herbicide. 2,4-D sprays have been particularly effective in controlling dicotyledonous weeds in cereal crops. Several million acres of crops are now sprayed annually by ground rig or airplane (16, 305). Among the hundreds of troublesome weeds and plant pests that can be controlled by 2,4-D, 2,4,5-T, or combinations of these, are bindweed, dandelion, plantain, chickweed, pigweed, woodsorrel, knotweed, broadleaf dock, shiny pennywort, honeysuckle, blackberry, Osage orange, guava, lantana, Java plum, and the tropical weeds "Aroma marabu" and "guao" (15, 136, 137, 138, 150, 151, 162, 208, 224, 226, 258, 274, 286, 322, 323).

Considerable attention has been given to herbicidal weeding of potatoes. This crop is relatively insensitive to 2,4-D (111) although yields may be reduced (89, 273). Other reports indicate that 2,4-D can be used for controlling weeds in potatoes without yield reduction (40, 105). Abnormal vegetative development and scab-like lesions on tubers result from spraying with 2,4,5-T (111, 331) or methyl α-naphthaleneacetate (273). Satisfactory control of weeds in gladioli, daffodils, and Dutch iris has been achieved with 2,4-D (171, 185).

Dinitro-*o*-cresol and related compounds also have been widely used in cereals, flax, and onions (156, 158, 194, 320). Although 2,4-D has largely supplanted the substituted nitrophenols on small grain in the United States, this

does not seem to be the case in Great Britain. Postemergence spray applications of 2,4-D have not been widely adopted on corn and other intertilled crops, partly because of some injury to certain varieties and partly because adequate weed control is usually not achieved.

The use of highly active herbicidal dusts is practical only under special conditions, because the usual formulations are easily carried considerable distances by wind with consequent danger of injury to nearby or even distant vegetation. Sensitive crops have been injured also by the drift of fine droplets of spray, either from airplane or ground-rig (46, 174, 255, 278).

With the introduction of herbicidal agents of low aqueous solubility and the use of low volumes rates, manufacturers have been confronted with serious problems of formulation. On the whole it has been found preferable to market liquid preparations that could be appropriately diluted by the user. One of the first 2,4-D preparations placed on sale consisted of the acid in a dry state admixed with excess of sodium carbonate; on solution the somewhat more soluble sodium salt was formed. It was later found, however, that maximum responsiveness is not obtained from alkaline solutions of 2,4-D (135, 199, 201). The incorporation of surface active agents may enhance appreciably the efficacy of aqueous herbicide formulations (110, 163, 219, 250, 277, 296, 338). The increased effectiveness in some cases may be due in part to the hygroscopic nature of the materials, which tends to maintain the active ingredient in solution for a longer period (119, 165, 250). In addition, surface active agents may function as co-solvents for substances of low water solubility, thus permitting the preparation of solutions of higher concentration. They are useful also as stabilizers in water-oil emulsions.

The alkanolamine salts of 2,4-D are reasonably soluble in water. The triethanolamine salt has been used in many formulations, usually with the addition of a wetting agent. Some companies have preferred to manufacture the esters of 2,4-D or 2,4,5-T which can be incorporated into oil emulsions and fortified oil sprays. Experience with a considerable variety of formulations and combinations has been reported (52, 74, 82, 84, 141, 160, 163, 165, 183, 207, 231, 257, 287, 295, 296, 303, 304, 315, 320).

Prevention of seedling establishment.—To prevent the establishment of weeds in a crop, herbicides can be applied to the prepared soil either shortly before planting (preplanting treatment) or, as is more common, after planting but prior to emergence of the crop (pre-emergence treatment) (232). In preplanting weed control, application of the herbicide is followed by seeding with minimum disturbance of the soil. Pre-emergence treatment holds great promise where the seed or seed piece is planted at a depth of an inch or more but is less useful for shallow sown or slowly germinating species.

Cyanamid and uramon have been used fairly extensively in preplanting preparation of seed beds, particularly for tobacco (330). These compounds and others, including ammonium sulfamate, ammonium thiocyanate, allyl alcohol, and 2,4-D have been employed in the preparation of seed beds for grass (96). Preplanting treatment with 2,4-D for fall planted spinach has

given satisfactory control of weeds and normal stands when the crop was planted 12 days after treatment (88). Aromatic naphthas are noneffective as preplanting herbicides (154).

Pre-emergence treatments may be subdivided into two types: (a) contact treatments, which employ light dosages designed to kill only the young weed seedlings that have emerged prior to the crop, and (b) residual treatments, in which the herbicide is desired to remain active in the soil for a time in order to suppress later germinating weeds. The latter type has also been termed selective soil sterilization. Perennial weeds, which develop from rhizomes or rootstocks, ordinarily are not seriously affected by pre-emergence treatments. Certain of the principles involved in the use of herbicides in pre-emergence weed control have been discussed (26, 77). 2,4-D affects germinating seeds and seedlings but not dormant seeds (218); this probably holds true for certain other herbicides as well.

Many types of herbicides have been tested in pre-emergence treatment. Spraying with oils frequently is an effective means of reducing the amount of early weeding. However, climatic conditions at the time of treatment may greatly influence the results (8, 154). Usually 50 to 150 gallons of oil are applied per acre. The oils have been used primarily on vegetable crops. Various types of oil, including Stoddard Solvent, diesel oil, fuel oil, and certain aromatic naphthas have been employed (8, 154, 189, 283, 307, 327); the choice is frequently determined by availability and cost (85).

Cyanamid has given good control of weeds in potatoes (170), in asparagus (307), in onions (155), and in corn (328). As rather high rates of this compound are required to control weeds, its use probably will be restricted to locations where the added nitrogen of the cyanamid is of particular value. Trichloroacetic acid and its sodium and ammonium salts control Bermuda and quackgrass when applied at rates of 80 to 100 pounds per acre, and Johnson grass at 100 to 150 pounds per acre. Annual grasses can be controlled by 25 to 40 pounds per acre. The use of trichloroacetic acid as a pre-emergence treatment does not appear generally promising because of injury to many crops, including corn, red clover, flax, and lima beans (202).

Although 2,4-D has been used for only a short period of time, many studies have been made of its effectiveness in pre-emergence treatments and a large acreage is so treated. Successful use requires careful timing and control of dosage rates and is dependent upon such factors as the nature of the crop and of the weeds, soil type and fertility, temperature and moisture conditions, especially pattern of rainfall distribution following application, depth of planting, and herbicide formulation (11, 53, 126, 245).

Successful pre-emergence treatment of corn with 2,4-D was first reported in 1947 (12); a large number of subsequent investigations have been summarized (126). The nature of the formulation of 2,4-D apparently does not significantly influence the results, although certain formulations may be preferable because of desirable physical properties. In general, rates of $\frac{1}{2}$ to 4 pounds of 2,4-D per acre were noninjurious to corn. Where 2,4-D was used

without subsequent cultivation, yield reductions were noted in all but one trial. Delayed pre-emergence application was more effecive than spraying at planting time. Similar results have been reported by others (11, 245). Pre-emergence weed control in certain other field crops (small grains, sugar beets, sweet clover, alfalfa, flax, soybeans, sorghum, peas, and bromegrass) was, for the most part, unsatisfactory in the north central region of the United States during 1948; in most instances there was only partial weed control together with some injury to the crop (53). Serious injury was caused to potatoes (170) and red beets (307) by 2,4-D, to onions by the isopropyl ester (310), and to a large number of other vegetables by the butyl ester (8). 2,4-D gave promising results in asparagus, lima beans, and in onions on peat soils (307).

Several dinitro formulations have been employed successfully though in some instances control of weeds, particularly grasses, has been poor (51, 87, 245); injury to corn has been noted. Large-seeded crops are tolerant of pentachlorophenol and dinitro-o-sec-butylphenol but small-seeded crops are not (29). Sodium pentachlorophenate gave promising results in pre-emergence treatment in lima beans (307), but was less effective than 2,4-D in onions (22) and in red beets while producing less injury to the crop (307). The use of pentachlorophenol and its sodium salt as an "activator" in pre-emergence sprays of ester formulations of 2,4-D has been suggested (140). Herbicidal activity of the combination persists longer than that of 2,4-D alone. Aromatic oils fortified with various phenols have been recommended for pre-emergence control of grasses in sugar cane and corn (77).

IPC also offers some promise for the pre-emergence treatment of grasses in certain crops (202).

Persistence of Herbicides in Soil

The persistence of herbicides is an important factor in determining their usages. Where complete eradication and prevention of reinfestation are sought, permanence of herbicidal activity is desirable. At the other extreme is the contact type pre-emergence treatment which requires minimal continued activity. Greater persistence, in various degrees, is wanted for pre-planting, residual type pre-emergence, and selective postemergence applications; at longest, the activity should not remain for more than one cropping season.

Partial or complete reduction of herbicidal effectiveness may result from leaching, from chemical reaction with soil constituents, from hydrolysis or decomposition through the action of microorganisms, from absorption or fixation by soil colloids, and, in particular cases, from volatilization of the agents (98, 252).

The disappearance of 2,4-D and related compounds from soil is due primarily to the action of soil microorganisms (49, 99, 210), and many of the environmental factors which influence persistence do so primarily as they affect the activities of the microorganisms concerned. In the amounts reach-

ing the soil in the usual herbicidal applications, phenoxyacetic acids do not affect adversely the activities of many kinds of soil microorganisms, including nitrifiers and nodule bacteria (60, 228, 242).

The effect of soil moisture and of soil temperature on persistence has been studied (49, 99, 176, 184, 222). The optimum soil moisture level for decomposition of 2,4-D, 2,4,5-T and Methoxone appears to be near saturation, 80 to 100 per cent of the water-holding capacity, and the optimum temperature is 30°C. or higher. Persistence was found to be increased by addition of lime and decreased by addition of large quantities of leaf mold (184); the addition of 1,000 to 4,000 pounds of manure per acre to a soil low in organic matter accelerated inactivation, while 8,000 pounds was without effect (49).

2,4-D is adsorbed by Norit A and by ion exchangers (200, 312, 313). As these adsorbents are not strictly analogous to the inorganic and organic exchange complexes of soils, the results are not directly applicable to agricultural soils. 2,4-D is less effective in muck than in mineral soils (185) perhaps owing to greater adsorption by the organic than the inorganic components. Leaching readily removes 2,4-D from some soils but not from others (78, 98, 142, 210, 233).

Under field conditions the persistence of 2,4-D has been variously reported as from less than 10 days to as long as 6 months (87, 98, 99, 151, 154, 289, 308, 313). There is disagreement also as to the relative persistency of 2,4-D, 2,4,5-T, and Methoxone (98, 313). These diverse findings are undoubtedly due in part to differences in the soil environment and in part to unequal treatment rates. In greenhouse experiments the persistence of 2,4-D applied at rates of 2.5, 10.0, and 50 mg. per pound of soil was 8, 11, and 21 days respectively (230). On retreatment of the soil with 2,4-D the period of persistence was reduced appreciably. This suggests that pre-emergence treatment of cropland with 2,4-D if repeated annually might become less effective for weed control.

The persistence of IPC under field conditions is of the order of one to three months (98, 289, 326). Its breakdown appears to be microbial (229) and factors that influence microbial activity affect its persistence. Trichloroacetic acid persisted in soil 30 to 60 days or more depending on rainfall (102). Toxic concentrations of dinitro-o-cresol or dinitro-o-sec-butylphenol are not attained in soil as a result of the usual spray applications (73, 79) and no evidence of accumulative or permanent toxicity was found to result from repeated additions of related phenols to soil (29). It is presumed that oils such as Stoddard Solvent are lost by volatilization and that nonvolatile oils are decomposed by soil microorganisms.

EQUIPMENT

The types of equipment used in applications of herbicides are to a great extent determined by the choice of the agent and the circumstances of its distribution. Where eradication of all vegetation is required and a high volume rate is acceptable, equipment similar to that employed for distribution of fungicides may be used. In general, such equipment operates at relatively

high pressures and has rather coarse nozzles. The contact herbicides are applied at substantial volume rates, even when used in a differential manner, such as for control of weeds in onions.

The introduction of translocated herbicides, such as 2,4-D, that are effective at low volume rates, has altered radically the specifications of spray equipment and widened the usefulness of this type of herbicide. The consensus of opinion favors use of the minimum volume rate necessary to secure adequate coverage. Much work remains to be done on optimum droplet size of translocated herbicides. According to preliminary studies, sprays of large droplet size are more effective than those of small droplet size due to greater interception of the spray on the foliage (272). High pressures are undesirable at low volume rates because of the probability of producing fine spray droplets that may drift away from the area being treated and perhaps cause injury to adjacent vegetation. For example, in a 3 m.p.h. wind a water droplet 5 microns in diameter can drift over three miles while falling 10 feet (43).

The choice of equipment depends in large measure upon the crop to be treated and the topography of the land (6, 25, 145, 204). An excellent description of herbicide spray equipment is available (7). For experimental field work the knapsack type is widely used and may be mounted on bicycle wheels carrying a small boom for applications to small plots. The pressure is produced by means of a cylinder of liquified carbon dioxide or compressed air or by a small gasoline motor and rotary pump (7, 54, 187, 263, 276, 329).

Many larger power rigs operating at 50 to 100 p.s.i. pressure and carrying booms up to 60 feet in length have been constructed. These may be self-propelled, with an independent power source for the pump, or be tractor-drawn (7, 44, 124, 187). Other models are mounted directly on a tractor, the pump being driven from the power take-off (6).

New equipment has been designed for dispensing herbicides in a fog, fine mist, or spray for special purposes (139, 279). For field studies, when volume rates and spray characteristics are under investigation, a modified DeVilbiss paint gun, Type CV or MBC, fitted with MBC-231 combination sprayhead and veiling cap, has been found useful at relatively low pressures (108, 272).

As early as 1940, Westgate & Raynor (320) reported on the use of aircraft for spraying Sinox in cereals and flax. Since then aircraft spraying and dusting has increased, particularly for weeds in rice (101, 258, 300), but also in many other crops (134, 311). Some of the problems involved have been discussed and recommendations published (45, 212). Large acreages of cereals were sprayed from the air in the U. S. and Canada in the 1948 and 1949 seasons. Aerial dusting of 2,4-D has now been prohibited in the U. S. by the Civil Aeronautics Authority because of the danger of damage to adjacent vegetation.

More recently the helicopter has become an important item of equipment for disseminating herbicides in inaccessible areas, particularly over mountainous terrain (237, 325). It has found use on the Pacific Coast, on waterways along the Gulf Coast, and in Great Britain. The strong down-draft from the rotor aids in directing the spray on the vegetation.

Physiological Aspects of Herbicidal Use

Advances in the utilization of herbicidal chemicals have been due largely to empirical methods, while understanding of the underlying principles has lagged far behind. Many workers in this field have expressed the view that progress in discovering superior herbicides and in developing new applications may be expedited greatly by knowledge of the physiological factors involved. Unfortunately, relatively few rigorously controlled physiological studies have yet appeared and dependence must be placed to large extent upon the circumstantial evidence available from field experimentation.

Entry.—With the exception of a few compounds, like sodium chloride, which act osmotically, herbicides to be effective must gain entry into the plant. Many of the cases of specificity, both as to herbicide and plant species, no doubt relate to the factor of entry into either the foliage or the root.

Materials are absorbed by roots principally as ions from an aqueous environment, so that in soil applications water-soluble and ionizable herbicides might be expected to be more effective than relatively insoluble and nonpolar compounds of equal intrinsic activity (76). However, many nonpolar and slightly soluble herbicides can be absorbed in lethal or toxic quantities by roots, e.g. esters of 2,4-D and related compounds (297), IPC (9, 10, 107, 288, 294), oils (181), carbon disulfide (143); the last presumably can be absorbed from the gas phase of the soil.

The factors influencing uptake by the foliage are more complex. Certain of the more corrosive herbicidal chemicals may so injure the superficial tissues of the leaf that entry is effected directly into deeper cells. Most of the herbicides now commonly employed, however, appear to penetrate through the intact surface by (*a*) diffusion as a vapor through the stomates, (*b*) mass movement as a liquid through the stomates, or (*c*) diffusion through the cuticle and epidermal walls.

Diffusion of gases through stomates might be expected to be an effective means of entry, although ordinary methods of herbicide application do not favor the maintenance of high vapor concentrations. However, stomatal entry might account for the reported superiority of volatile esters of 2,4-D over the non-volatile acid and salts (76, 83) and of the more volatile ammonium salt of dinitrocresol over the sodium salt (33, 119).

The stomata appear to be important, though not exclusive, portals of entry for herbicidal oils, and particularly for those of low surface tensions (181, 253, 298, 301, 333, 334). Aqueous solutions do not ordinarily penetrate open stomata (67, 119, 256, 266, 301, 321, 337), and they are not of importance in entry of 2,4-D from such solutions (296, 314), though the addition of oils or surface tension depressants may sometimes permit entry.

Diffusion through the cuticle is probably the usual means of entry. The amount which enters is a function of the time and area of contact; both factors are related to the degree of wetting of the surface and the contact angles of discrete droplets, if present (118). The surface roughness of the leaf, which may affect both retention of droplets and the contact angles established, is susceptible to change in response to environmental conditions

(117, 118, 120). Positional and species differences in wettability of leaves have been noted (103, 118), but these have not been correlated with studies on herbicidal entry and effectiveness. The role of leaf hairs in entry merits further investigation (110).

The nature of the active agent may be a factor in entry. As the cuticle of leaves is largely lipoidal, it has been surmised that nonpolar molecules penetrate much more readily than polar (76, 83). Unambiguous evidence in support of this view is lacking, however, while the effectiveness of aqueous sprays of polar herbicides such as sodium chlorate, ammonium sulfamate, and 2,4-D demonstrates that such substances can penetrate the cuticle. Of greater importance in the entry of polar compounds appears to be the extent of dissociation. Weak acids in general are absorbed by living cells in the form of the undissociated molecules rather than as ions, perhaps owing to their greater lipoid solubility. Many herbicides are salts of weak acids. Acidification, which decreases dissociation, enhances activity. Thus, "activation" of Sinox and of pentachlorophenate is brought about by addition of acid or acidic salts (84, 146, 235), toxicity of chlorate is greater at low pH (1, 24, 69, 167, 169, 239, 332), acid arsenical sprays are more effective than alkaline (68, 173), and response to 2,4-D is greater at low than at high pH (135, 199). In acidified solutions the buffer capacity as well as the pH may be of importance (135, 201); perhaps in order to counteract the neutralizing action of the plant tissues.

Translocation.—Herbicides which gain access to superficial cells of the plant in no case remain entirely restricted to these cells. Various materials differ greatly, however, in the degree to which they move in the plant, certain substances being virtually confined to the organ that they enter while others become more or less widely distributed. The older classification of herbicides into "contact" and "translocated" types reflects such differences although various meanings are now attached to these terms. An intimate relation exists between translocation and mode of herbicidal action as, on the one hand, the extent of distribution of the chemical through the plant determines which tissues can be directly affected while, on the other, the initial toxic effects of the herbicide may determine whether transport can readily occur. Rapid killing of superficial cells (e.g., by phenols or copper salts) may tend to restrict further movement, whereas more slowly acting compounds such as sodium chlorate and 2,4-D become widely distributed. In the former case the organ which first comes into contact with the herbicide may be killed, while other portions of the plant continue to grow (41, 119); with the more extensively translocated compounds the opportunity for regenerative development from uninjured parts is greatly diminished (259).

There appear to be several, more or less distinct, mechanisms of transport of herbicides in the plant. All water-soluble substances will spread to some extent by diffusion but the velocity of this process is so low that it may be presumed to play a very minor role in herbicidal action. Substances which penetrate the root and enter the transpiration stream are moved upwards in it. Conduction in this manner takes place through the xylem, is dependent

upon those environmental factors which influence transpiration, and may attain relatively high velocities (114, 161, 198, 267). If toxic chemicals are applied to a leaf, the direction of water movement between it and the shoot may be reversed so that the applied material is carried basipetally. Sodium chlorate sprayed on the upper leaves was observed to produce injury to the lower leaves but not to that portion of the plant below the lowest transpiring leaf (198); the roots are not killed and resprouting occurs (70). Arsenical foliage sprays may kill roots to a depth of several feet (130, 131). Rapid movement of herbicide from leaf to root in this case is believed due to mass movement of spray liquid into vessels in which a water deficit has been created by conditions favoring low root absorption and high transpiration loss (68).

Although xylem transport may on occasion be involved in the movement of the hormone-type herbicides (64, 288, 317), distribution depends ordinarily on a quite different mechanism to which their efficacy in spray applications is in large measure attributable. Unlike those chemicals which exert a more or less direct toxic action on protoplasm, and hence are precluded from transport mediated by the normal activities of living cells, the exogenous growth-regulatory compounds appear to be conducted via the phloem by the same mechanism which governs translocation of endogenous metabolites (317). In consequence, they move, with a minimum of initial damage to conducting cells, into all those tissues of the plant which are actively growing and which at the same time are most vulnerable to their action. Export of growth-regulators from the leaf in this fashion appears to depend on concurrent movement of elaborated foodstuffs and hence is most rapid under conditions favoring photosynthesis (81, 193, 217, 250, 314).

A still different mechanism is responsible for the transport of oils in plants. Although movement may take place in the vascular system in some plants (181, 333, 334, 335) it is not confined thereto and is apparently more or less independent of that of aqueous solutions, with which the oils are immiscible. Capillarity is believed to play an important role (253) and there has been proposed an "oil-mass theory" which postulates that petroleum oil coalesces with lipoidal and other oil-miscible portions of the cytoplasm to form an extended continuous oil phase, the movement of which in the plant is assisted by capillarity, gravity, and the bending of tissues by wind (334). With respect to the rapidity and extent of movement of oil through tissues and into cells, there appear to be marked differences among species and among tissues of a particular species as well as among various types of oils (153, 181, 214, 253, 334).

Mechanism of action.—The manner in which herbicides bring about the death of plants appears to depend upon the particular chemical concerned. In no case has a complete and detailed description of the causative sequence of events been proposed. Information is available as to a number of physiological, biochemical, and morphological derangements which may attend the action of herbicides but, for the most part, it is not clear whether these are to be regarded as causes of death or as incidental responses. The cellular re-

actions to high concentrations of various types of herbicides are nonspecific (86).

The best understood mechanism is that of substances, like sodium chloride, that act osmotically. This requires an external solution of osmotic pressure greater than that of the plant cells, which for many mesophytes falls within the range of 5 to 20 atm.

A number of heavy metals are known to combine with or precipitate proteins, enzymes, and other cellular constituents. Similar effects may be presumed to be brought about by herbicides such as copper sulfate and iron sulfate, although there is no direct evidence that this is the cause of their lethal action. Marked cytoplasmic derangements are caused by moderately high concentrations of copper salts (260). Copper chloride has been reported far superior as a herbicide to copper nitrate which in turn is more effective than copper sulfate (33) but it is not known to what extent this is related to the end action of these salts rather than to the process of penetration.

Trichloracetic acid also is an effective protein precipitant, but what role this property plays in its herbicidal action has not been established.

Dinitrophenol has been shown to act on the basic cellular mechanism by which phosphate bond generation is linked to oxidation in such a way as to uncouple these reactions (196). As a consequence synthetic reactions are blocked, phosphate uptake inhibited, and growth and differentiation prevented. At the same time respiration may be unaffected or actually increased. Although all these effects have not yet been specifically demonstrated in higher plants, it seems quite probable that the action of herbicidal phenols will prove to be the same (37, 177, 235, 244).

The toxic action of sodium chlorate may be due to a decomposition product rather than to the applied chemical itself. The chemical similarity between nitrate and chlorate, the specific inhibition of chlorate toxicity by nitrate, and the correspondence between chlorate injury and nitrate reduction with respect to influential conditions and localization in the plant have led to the hypothesis that chlorate is reduced by the cellular mechanism which ordinarily reduces nitrate (1). The putative products, chlorite and hypochlorite, are regarded as the actual toxicants. Hypochlorite is thought to bring about rapid denaturation of proteins; the action of chlorite *in vivo* is unknown but it may be of significance that this compound *in vitro* decomposed many cellular constituents, including lignin. In this connection it is of interest that disappearance of starch grains and disintegration of cell walls has been observed following chlorate treatment (192). The rapid blackening of tissue by chlorate has been ascribed to oxidation of respiratory chromogens (148). Respiration may be increased in chlorate-treated plants (23, 246).

Arsenite and arsenate are potent protoplasmic poisons presumably owing in large measure to their activity as inhibitors of sulfhydryl enzymes. Antagonism between phosphate and arsenate has been observed (168) but the phosphate status of plant or soil does not appear to have been studied as a factor modifying herbicidal effectiveness of arsenicals.

Two types of toxicity of herbicidal oils have been distinguished. "Acute" toxicity, which is due to the more volatile aromatic and olefinic petroleum constituents or their peroxidic and acidic oxidation products, is manifested by rapid burning of the foliage and may lead to early death; the more slowly appearing "chronic" injury is caused by the higher boiling constituents (61, 85, 97, 132, 152, 153, 172). Even though certain types of cells are able to withstand prolonged immersion in purified mineral oil (20) the presence of considerable amounts of oil in intercellular spaces and tracheae might be expected to be inimical to normal cell functioning through interference with gaseous diffusion (333) and water movement (214). Rapid diminution of photosynthesis and transpiration has been observed to follow treatment with oil; decreases in respiration have been observed in some experiments and increases in others (172, 178, 181, 214, 235). With the less toxic oils, or in resistant species, these processes subsequently may return to normal. Certain oils bring about cellular disorganization (4, 253).

2,4-D has been shown to affect many plant processes including photosynthesis (123), water relationships (39, 48), stomatal behavior (39, 115), and nutrient ion absorption and accumulation (227, 249, 271). The respiration of entire plants and plant parts may be markedly influenced by 2,4-D (48, 91, 123, 166, 177, 215, 246, 270, 271, 290). The nature of the response is dependent in part upon the concentration of the growth regulator. Extremely low concentrations cause a transitory stimulation in carbon dioxide evolution; intermediate concentrations produce a permanent increase in respiratory rate which may continue until the plant or tissue dies; and high concentrations may bring about a markedly subnormal rate of carbon dioxide evolution which is sometimes preceded by an initial stimulation. Stimulation is greater in starved than in unstarved tissues (177). Pea stem segments are more sensitive to 2,4-D than are oat coleoptile segments (177).

Carbohydrate metabolism is frequently affected (144, 216, 221, 246, 270, 271, 280, 299). In general, sugars increase and starch and dextrin decrease immediately after treatment. Subsequently all three fractions diminish. The sugars presumably arise from the hydrolysis of starch and dextrin and the ultimate decrease of all available carbohydrates has been attributed to their utilization in respiration. It has been suggested that depletion of carbohydrates is a factor resulting in the death of plants which have been treated with 2,4-D (216) but this view has been challenged on the grounds that abundant carbohydrate is still present when death occurs (246, 270). Interference with normal phloem function and food transport have been suggested as the proximal cause of death (270, 299).

The action of IPC appears to be related primarily to its effect upon the mitotic process and cell division. Young cereal plants and certain susceptible broadleaf plants treated with the substance exhibit abnormal cytological behavior characterized by an interrupted mitotic axis, blocked metaphases, multinucleate cells, occurrence of giant nuclei, and highly increased chromosome number in certain cells of both root and shoot. Generally, cell division is arrested and those cells which are capable of further growth enlarge greatly (100, 108, 109).

HERBICIDES OF THE FUTURE

The contribution that can be made to agricultural and horticultural production by the efficient use of herbicides is difficult to assess. Through competition for nutrients, for water, and for space, the growth of crops, particularly when young, is adversely affected by weeds. Estimates of the extent of yield reduction caused by weed competition, that are based on comparisons with control plots in which weeds are removed by hand or by cultivation, are not wholly valid. There is little doubt, however, that significant increases in total production of all major crops could be achieved by effective measures of weed control. It has been estimated that widespread adoption of 2,4-D for weed control in the United States could increase grassland utilization by 25 per cent and grain yields from 15 to 100 per cent, depending upon the degree of weed infestation (305). The outstanding development of the last few years has been the employment of herbicides and herbicidal practices for weed control in the low-value crops, such as the cereals that bulk so large in overall production. Important as are the horticultural applications, they may be less significant ultimately than the agronomic applications.

Future progress in use of herbicides may be expected along three lines: (a) discovery of superior compounds; (b) development of improved combinations and formulations of herbicidal substances; and (c) wider utilization both in current types of practice and in hitherto undeveloped directions.

Selectivity.—With the exception of a few specialized applications where eradication of all plant growth is desired, the great advantage of recent developments in chemical methods of weed control lies in their ability to kill or severely damage some plant species while leaving others practically unharmed. In present research, compounds are being sought, developed, and formulated for specific uses, such as the control of crabgrass in lawns, or the elimination of weeds in carrots. The reasons for such selective action consequently are of the greatest interest. There is no clearly predictable basis of selectivity although certain factors, which under various circumstances may contribute to selectivity, have been recognized (74). One or more of these may be operative in any particular case. Selectivity in many cases appears to be related to developmental and morphological factors such as shape and position of leaves, depth of distribution of root system, thickness and composition of cuticle, number and characteristics of stomates, degree of pubescence, and protected or exposed location of growing points. Usually the differences between susceptibility of crop and weed is one of degree only. In general, plants become more resistant to herbicides as they become older and larger. Species differences may, therefore, be accentuated by differences due to age or to the physiological stage of development. Perennial species, when dormant, may be unaffected by treatments which would be lethal during a period of vigorous growth. Whether there exist among various kinds of plants differences in herbicidal sensitivity at the cellular level, as is suggested by some experiments, cannot at present be stated.

One important problem yet to be solved is the discovery and development of herbicides that can efficiently control annual and perennial grassy weeds. 2,4-D and related compounds can be used in a selective manner to

remove dicotyledonous weeds from cereal plantings, lawns, or pastures, but at present there is available no satisfactory means of accomplishing the converse result. IPC, which at first was hailed as a "grass-killer," has not been found generally effective for this purpose.

The search for new herbicides.—The diverse and often spectacular results obtained in weed control by the use of 2,4-D have given impetus to the search for new herbicides. Various groups and laboratories are engaged in the routine testing of candidate compounds. The Chemical-Biological Co-ordination Center of the National Research Council includes the results of phytotoxicity tests in the data on biological activity of all types which it collects and collates on compounds in its files.

At present, selection is almost wholly empirical. Some, and perhaps many, compounds may be found to possess toxicity in more than one plant function or tissue activity. The full recognition and description of the herbicidal characteristics of chemical compounds may point the way toward more effective combinations. The most acute problem which confronts those engaged in the search for potential herbicides is the development of appropriate and adequate routine tests. The amounts of the compounds initially available may be small. The circumstances of herbicidal use are diverse in character, and complicated by the newer and more specific requirements of selective use or differential action. Final proof of effectiveness can be obtained only by actual field experiments, properly designed and conducted. These may have to be repeated for several seasons in numerous localities. The recent herbicide literature contains many examples of premature publication and inadequate field experimentation.

Routine screening procedures have been based primarily on the observation of responses produced in greenhouse-grown plants treated in a variety of ways. Many of the tests recently employed have been intended to detect activity akin to that of the phenoxyacetic acids with the danger that compounds producing wholly different types of plant responses may be overlooked. Arbitrary choice of concentrations of the test compounds is often made. Wherever possible it seems more desirable to include a range of concentrations sufficient for determination of the shape of the dose-response curve, whence activities of different compounds can better be compared (50, 127). Standardization of test solutions with respect to pH, co-solvent, wetting agents, etc., is highly desirable (50, 164, 213).

A group of British workers were probably the first to investigate selective phytocidal action of compounds by a variety of tests (268) in which extent of kill, reduction in germination, and suppression of shoot and root elongation of various species were used as criteria. Inhibition of growth, as compared with that produced by a standard dose of 2,4-D, was used in screening over 1,000 compounds for herbicidal activity (297). Responses studied were inhibition of primary root elongation of germinating corn (281), inhibition of shoot growth of kidney beans following the application of the test material in a single aqueous droplet (219), and a similar application in oil (282). Cucumber seedlings have been shown to be more satisfactory and sen-

sitive than corn in germination studies (248); others have used mustard (34, 268), or cress (17, 18, 34, 268), or rice (47). A leaf immersion technique using corn and tomato plants has been described (179).

Methods involving the application of test compounds to the soil of potted plants have been widely employed for screening both hormone and non-hormone type herbicides (69, 72, 73, 161, 213, 252). Other methods have involved a great diversity of test objects and modes of application. Materials have been applied to seeds and aerial plant parts by means of dusts (183), aerosols (339, 340), sprays, both aqueous (84, 183, 206, 213, 316) and non-aqueous (282), single droplets (50, 219, 282, 297), lanolin smears (116, 183), carbowax pellets (219), injections (160, 251), immersion (179), submersion (13, 47, 248), and flotation (236).

The criteria of response also have been many and varied. The available quantitative data obtained have only relative value and cannot be treated like physical constants. Extrapolation from such tests to field use depends to a considerable extent on the experience of the worker. The screening tests merely permit the conclusion that a particular compound is phytotoxic, or inhibitory, or injurious under the test conditions. Its development and evaluation as a practical herbicide must start from that point.

The major emphasis of current developmental herbicide research appears to be on the discovery of compounds with selective toxicity. Less attention has been given to the preparation of compounds of a desired degree of persistence in the soil, a property which, as indicated above, may frequently be of considerable importance. Much more information is needed also concerning the physiological aspects of herbicidal action, better understanding of which may be expected to contribute greatly to its more intelligent employment.

Increased yield, perhaps apart from that due to elimination of weed competition, has occasionally been noted following the treatment of a soil or crop with herbicides (35, 56, 73, 78, 79, 142, 147, 289). There are reports also of desirable alterations of chemical composition, such as higher protein content in wheat, resulting from such treatments (3, 112, 220, 265). Should findings of this kind be substantitated by future research, it is possible that the use of herbicides may yield unexpected dividends.

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PHYSIOLOGY OF CELL WALL GROWTH[1]

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The advancement of cell physiology is intimately linked with our knowledge of cell morphology. As long as biochemists ignore morphological problems, their work must necessarily end in an impasse. It is not only important to determine the chemical processes in a given cell, but we must also know exactly in which cellular element a certain reaction occurs before we can come to a satisfactory understanding of any physiological phenomenon. In this respect the actual stimulation to the study of respiration by the discovery that respiratory enzymes are localized on the mitochondria (30) may be mentioned.

In the field of cell wall physiology, morphological study has always kept pace with biochemical research, or has even been more advanced, as the wall is such a conspicuous part of the plant cell and is endowed with characteristic anisotropic properties which make it an ideal object for the indirect research methods of submicroscopy (22). These investigations have led to the conclusion that the plant cell wall has a reticulate structure, which contrasts profoundly with the particulate organization of other colloids (15). Since a reticulate framework is a prerequisite of any gel structure (17), the cell wall is the prototype of a biogel. Therefore, a thorough morphological knowledge of the cell wall helps, not only to elucidate the physiology of the walls, but also provides some possibility of understanding the nature of the plasmagel and its mysterious gel-sol transformations.

Morphology.—Ontogenetically, two different layers can be distinguished in the plant cell wall; the primary and the secondary wall. The first is a thin membrane, while the second may become a thick wall with three or more layers (2). In the mature cell, the primary wall may be overlooked in view of the striking secondary wall, with its conspicuous morphological features, such as layers, striations, and pits. But physiologically the primary membrane is very important, because it is the only envelope of the young cell as long as the protoplast is growing. Only when the cell has reached its final size are the secondary layers deposited, making the wall solid and rigid.

The primary wall has the remarkable capacity of growing in surface by intussusception, while the secondary wall grows mainly in thickness by apposition. Chemically there is no fundamental difference between the two walls. Both consist of cellulose, hemicelluloses, and pectins, and both may become lignified. In general, however, the noncellulosic substances prevail in the primary wall whereas the secondary wall may contain almost pure cellulose (cotton hairs and certain bast fibers), or moderately lignified cellulose (conifer tracheids), and is therefore the source of cellulose for technical purposes.

[1] This review covers the period from approximately 1935 to 1949.

While there is no qualitative chemical difference between the two onto-
genetic layers, they display most striking differences in their physical be-
havior. The secondary wall can be split into layers, and the layers into
fibrillar strands (18) with isodiametric cross sections, e.g., of 0.4 μ diameter
in cotton. These strands exhibit a high anisotropy of swelling (20 per cent
perpendicular, and almost none parallel to the fiber axis), an astonishing
tensile strength (up to 100 kg. per sq. mm.), double refraction (up to 0.07),
and very conspicuous dichroism when stained with suitable dyes (13, 57).
The x-ray analysis yields interference spots revealing a high degree of orien-
tation of cellulose within the secondary wall (3, 43, 44, 53).

The primary wall, on the other hand, cannot be split into thinner sheets
or fibrillar strands, its anisotropy of swelling is small, its tensile strength in-
significant, and when torn apart it tears in quite an irregular way. Its optical
anisotropy is low, and x-ray spectrographs show rings indicating a poor
orientation of crystallized cellulose (14).

From all these facts, it was concluded (14, 22) that in the primary wall
there must be a "scattered texture" (in German "Streuungstextur") of
submicroscopic cellulose strands, while the secondary wall is characterized
by a "parallel texture" (in German "Paralleltextur"). The parallelized
strands may run strictly in the direction of the cell length forming the clas-
sical fiber texture, or they may form an angle with the cell axis, as in cotton
or wood fibers, and yield a spiral texture.

In the primary wall the scattering may be complete, in which case the
membrane is statistically isotropic when observed perpendicularly to its sur-
face. This arrangement has been termed "foliate texture;" it is formed in
isodiametric meristematic cells. When such cells elongate, the polarizing
microscope reveals a certain predominance of strands running transversely
to the axis of elongation. The scattering is such that the orientation in the
transverse direction predominates over the orientation parallel to the cell
axis. This arrangement is called "tube texture," as it is characteristic of
tubular cells.

In cell walls with tube texture the angle of dispersion of the cellulose
strands can be calculated (19, 20). The dispersion of the strands may be
idealized by assuming that cellulose strands occur at angles uniformly distrib-
uted about the transverse direction, up to an angle α, called the angle of
distribution. Strands with a steeper angle than α would not occur. The cal-
culation of this angle is based on measurements of iodine dichroism or double
refraction. With chlorozinc iodine parallel-textured cellulose walls yield the
dichroism black violet/colorless in the polarizing microscope, while scattered
strands show an anisotropy of absorption dark violet/faint violet. When the
dispersion is complete (angle of distribution $=90°$), the dichroism disappears
entirely. When the coefficients of absorption of iodine-stained cell walls are
measured parallel ($k_{\parallel}$) and perpendicular ($k_{\perp}$) to the cell axis the di-
chroism is $k_{\perp} - k_{\parallel}$, and for the angle of distribution α we find

$$\frac{k_{\parallel}}{k_{\perp}} = \frac{1 - \cos \alpha}{\sin \alpha}.$$

For the double refraction the relation is

$$n_\perp - n_\parallel = (n_a - n_o) \frac{\sin 2\alpha}{2\alpha},$$

where $n_\perp - n_\parallel$ is the double refraction of the cell wall with tube texture, $n_a - n_o$ the double refraction of a corresponding parallel texture, and α the angle of distribution. For latex cells of *Euphorbia splendens*, which have tube-textured secondary walls, these formulae yield angles of distribution from 60° to 67°.

It is a very welcome development that all these textures calculated from results with indirect research methods (polarization microscopy, x-ray analysis) can be seen today in the electron microscope (26, 38, 45). It turns out that primary and secondary walls have a framework of cellulose strands with an astonishingly constant diameter of 250 to 300 Å. In the secondary wall of cotton and ramie fibers, *Valonia* cell wall, etc., the cellulose strands are arranged strictly parallel (parallel texture), while in primary walls (corn root, oat coleoptile) they show a conspicuous dispersion. Besides thus verifying the scattered textures, the electron microscope has shown that the cellulose strands in primary walls are interwoven, which explains why they cannot be split into thinner membranes like the parallel-textured secondary walls. For these, the existence of cellulose strands, termed "microfibers," of about 250 Å diameter had been predicted (16), but for primary walls thinner strands had been expected. Seemingly the cytoplasm builds cellulose filaments of a constant thickness and uses these microfibers to construct all kinds of different fabrics and textures. It is interesting that in the cellulose gel of *Bacterium xylinum*, which is formed outside the cell, cellulose strands of uniform diameter are also observed (25, 37, 39); but there is some tendency toward aggregation of those strands to form bands and twisted ribbons, while in cell walls the individuality of the strands is maintained in all textures observed to date.

From a molecular viewpoint, the cellulose microfibers discovered are rather large aggregates. Since a cellulose chain has a diameter of only 5 Å, strands with 250 to 300 Å thickness must contain 2,500 to 3,600 cellulose molecules. It is probable that not all of them are ideally crystallized, but that some parts have a perfect chain lattice and that other regions are of a less orderly arrangement, causing pronounced flexibility of these microfibers. Intensity measurements of x-ray diffraction pattern yield 70 per cent crystallinity for bast fibers (flax, cotton, ramie) and only 40 per cent for bacterial cellulose (26a).

Growth in surface.—The most interesting feature of the primary cell wall is its capacity for growing in area. This is a morphogenetic problem and, therefore, cannot be explained by mere mechanistic theories. Heyn (27) showed that during the growth of cell walls the plasticity increases. This leads to stretching of the wall by the turgor pressure within the cell, resulting in a balloon-shaped cell. Instead of this, the cell elongates, which must be ascribed to the fact that the tube texture of the wall has a greater tensile

strength transversely than longitudinally (14, 22). A uniform plasticizing of the membrane would further result in a uniform stretching of the whole wall, while actually the different faces of the cell, considered as a polyhedron (35), extend quite differently; it has even been observed that one part of a face may grow (e.g., forming a root hair), while the rest of the same face remains unchanged (8, 21).

When the expanded cell is plasmolized the cell wall does not assume its original shape; although it contracts, its surface remains larger than before. Thus an elastic and a plastic extension have been distinguished (27). Burström (8) does not agree with this view. He thinks that there is no plastic deformation, but that the nonelastic extension of the wall is growth by intussusception. This seems rather likely, as it has been proved that considerable synthesis of wall substances takes place during cell elongation (23, 59). These questions can be discussed today on the basis of electron micrographs which show the behavior of the texture of a primary wall during growth (26).

The elongating cell wall displays a loosening of the fabric of its cellulose strands. Since the strands are interwoven, this phenomenon cannot be a simple matter of plasticizing, e.g., by a change of hydration; rather some of the existing strands must be cut or dissolved to bring the wall into a semiliquid state and allow the texture to expand. It loses some of its tensile strength and extends in all directions. Afterwards new strands are woven into the loosened texture, and the original state is restored. This behavior of the wall would necessarily cause the cell to round off if the whole surface were liquefied at the same time. However, the electron micrograph shows that only a small area of a few μ in diameter is "plasticised," while adjacent areas maintain their dense texture during this process. Thus the growth in surface of the cell wall is probably localized in small areas, while the other parts maintain the solidity of the membrane. As soon as the expanded area has reassumed its original texture, other areas may be "plasticized" and give way to the turgor pressure. It is likely, therefore, that the growth in surface proceeds in a mosaic pattern.

The electron micrographs discussed above provide a basis for describing the surface growth of cell walls. The elasticity is due to the submicroscopic cellulose texture of the cell wall. The irreversible stretching of the wall is caused by local weakening of the texture. From a mechanical viewpoint this must be considered as a plasticizing process. But, as soon as the loosened wall yields, it is restored by the formation of new cellulose strands. This process is the actual mechanism of "growth by intussusception." It may be recalled that this double phenomenon of softening and restoring the wall texture was postulated many years ago (14), although it was then thought that the cellulose strands in the primary walls would be much thinner than they really are (only about 50 Å instead of 300 Å thick).

The possibility that the framework of the wall may be loosened in localized areas helps explain how a cell can produce an outgrowth (e.g. root hairs, pollen tubes, anastomoses of latex cells, etc.,) or restrict growth to the end of the cell (tip growth of fibers).

This explanation of growth in surface solves many old problems, but raises new ones. The local dissolution of submicroscopic cellulose strands requires the presence of the enzyme cellulase or cytase. It would be very interesting to detect cellulase in meristems, because it is lacking in mature plant tissues. Although its presence is needed for the phenomenon of cell fusion (vessels, anastomosing latex cells), its general occurrence in all growing plant cells has not yet been postulated. This enzyme must work only very locally, while in nearby areas the loosened textures are being rebuilt by synthesis of cellulose. Thus it must be accepted that the living cytoplasm which penetrates the growing cell wall correlates this simultaneous decomposition and rebuilding of the wall structure in some unknown way, which is called morphogenesis.

Tip growth of cells.—A serious problem is the question of how meristematic cells change their shape in a tissue during their differentiation into fibers, vessels members, etc. As far back as 1886 Krabbe (34) evolved the concept of "sliding growth." He believed that the middle lamella between adjacent cells was dissolved, allowing the cambial cells to slide along each other and grow out to long fibers, or widen to vessels, idioblasts, etc. This theory has been criticized by Priestley (46), who showed that the existence of pits between growing cells was incompatible with the dissociation and sliding of adjacent walls, because sliding would have to destroy the correspondence observed to occur between pits in adjacent cells. Priestley concluded, therefore, that neighboring cell walls must grow simultaneously at the same rate, which he called "symplastic growth." This view has been supported in an extensive monograph by Meeuse (36). When idioblasts or latex ducts are expanding in growing tissues and come into contact with cells which originally were not neighbors, the concept of symplastic growth is certainly not adequate. Therefore, Sinnott & Bloch (52) postulate the active penetration of a growing cell between others, which they term "intrusive growth." The best experimental evidence for this type of growth has been presented by Schoch-Bodmer & Huber, who show that flax fibers display a typical tip growth (50). The bodies of neighboring fibers are grown together and expand by symplastic growth, but the cell ends behave just like the tips of root hairs or pollen tubes (48). They are rounded, rich in protoplasm (much richer than the body of the young fiber), contain the nucleus, and protrude between adjacent fibers or parenchyma cells. The middle lamella of these adjacent cells is split, and the primary wall of the intruding cell tip lies against the existing walls of the cleaved and separated cells. If this intrusion meets an obstacle, the fiber tip is molded to the shape of the hindrance or it may even bifurcate (51).

This type of cell differentiation and wall growth within a tissue is of general theoretical importance. If the theory of sliding growth were true, the tissue would have to lose its entity and to disintegrate into individual cells. Each of these cells would grow and differentiate as a unicellular protophyte; only in the mature state would they reunite again to form a tissue. Seen in this admittedly extreme manner, such a differentiating meristem

would not represent a true tissue of a metabiont, but rather, a cell colony. On the other hand, pure symplastic growth is impossible in view of the enormous changes of cell shape during differentiation. As is often the case with conflicting theories, the truth lies between the two. In this case, parts of the cells (the body of the fibers) stay united during differentiation, assuring the entity of the tissue and allowing a correlated histological growth. On the other hand, the cell tips behave independently resulting in a cell shape which may differ from the shape of neighboring cells.

An instructive example of such differentiation is the formation of secondary phloem and xylem in *Sparmannia* (51). The fibers derived from the cambium elongate and the vessel members increase in diameter. The fiber tips intrude between neighboring cells, so that in a cross section the linear arrangement of the cambial derivatives is disturbed. This is especially striking with phloem fibers. In the cell file derived from a single cambial initial as many as four fibers may be found adjacent to one sieve tube. In the xylem the file of lignified cells derived from a single cambium initial is disturbed in a similar way but to a smaller extent. This is due to the fact that the tip growth of the phloem fibers is about twice as great as that of the wood fibers. During differentiation the cell-body of the cambial cells (termed by Schoch-Bodmer & Huber "Cambium-Mittelstück") maintains its histological relationship with the other cells, while the cell tips elongate. In this way pits between the cambial cell bodies are not disturbed during growth.

The differentiation of vessel members also occurs by local growth. Parts of the circumference of the cambial cells protrude in-between the adjacent cells, pushing them apart. In this way the linear files produced by a cambial cell undergo additional disturbance. In the parts of the cell wall which do not grow the pits remain, while the intruded membranes may form new pits in contact with a cell wall which is still differentiating.

The different phenomena of extension-growth, where the whole cell wall elongates, of tip-growth, where a new cell wall is formed by the cell end, of width-growth, where parts of a given cell wall increase in girth, as well as the possible combinations of these growth types, are clearly grouped by Schoch-Bodmer & Huber (51). The active increase of wall surface may cause intrusive growth, which these authors call "interposition-growth," because the newly formed cell wall is laid down against an already existing one (49, 51). This term is likely to illustrate the mechanism of intrusive growth.

The terminology of cell wall growth has become rather tedious: extension-growth, tip-growth, and width-growth for the observed phenomena; and sliding growth, symplastic growth, intrusive growth, and interposition-growth for the explanation of the observed facts. As seen from the point of view of submicroscopic morphology, all these different terms can be subsumed under the concept of growth in surface.

This growth in surface consists of a plasticizing of the cell wall and actual growth by intussusception. When both phenomena occur diffusely, there is extension growth; but if they continue for some time at the same spot, there is tip- or width-growth. In all cases, growth is restricted to lo-

calized areas. Whereas in extension-growth this localization changes with time, in tip- and width-growth it remains restricted to a certain area for a longer period. Symplastic growth would mean that two adjacent walls have the same rate of growth in surface, while intrusive or interposition-growth is due to a pronounced difference of intussusception in neighboring walls. If this difference is very great, an impression of sliding growth might occur, but a real individualization of the cells never takes place. The cells of the meristem remain always somehow interconnected, so that the tissue grows as a whole and a histological morphogenesis is assured.

Spiral growth.—Spiral growth is known not only of multicellular organs but also of individual cells (*Valonia*, *Chara*, sporangiophore of *Phycomyces*, cotton hairs). It has been studied with particular thoroughness in *Phycomyces*, where it was discovered by Burgeff (7), described by Oort & Roelofson (40, 41) and fully investigated by Castle (12). In the beginning, the elongation of the sporangiophore of *Phycomyces* is a typical tip-growth, in which a tapering terminal zone about 1 mm. long is involved. During this elongation the tip rotates following (according to mathematical terminology) a left-hand spiral with a slope of about 80°. Later, the sporangium begins to swell and becomes a big bowl, while elongation and rotation cease. After 2 or 3 hr. both reappear simultaneously and continue for many hours. The growth zone then lies below the inflated sporangium; it is 1.5 to 2 mm. long and still tapered. During the first hour of this stage, the growth follows a right-hand spiral of 75° to 83°. This rotation, however, slows down to zero and then reverses, now forming a left-hand spiral of 78° to 83°.

These intriguing facts are very difficult to explain mechanically. Nevertheless many explanations have been proposed, especially before the complicated reversals observed by Castle where known. Heyn (28) thought that the chain lattice of the crystallized chitin within the cell wall would induce a spiral slope of 76.5°. But, as the angle changes during growth, this suggestion fails to give a satisfactory explanation. Castle (10) suggested a model in which the tapering shape of the growing zone is involved in the production of rotation. As there are cylindrical cells with spiral texture (e.g., cotton hairs), v. Iterson (31) formulated the following three conditions which a mechanical explanation of spiral growth must fulfill: (a) the model must have cylindrical shape with originally no spiral texture; (b) the model must yield left as well as right-handed spirals of different slopes; and (c) the model must yield spiral growth by forces known in growing organisms. The model of v. Iterson satisfies these conditions. He postulates a texture parallel to the axis of the cylinder (fiber texture), enclosed by an elastic membrane. This corresponds to the organization of a fiber cell with its elastic primary wall and bulky parallel-textured secondary layers. If the secondary wall has a tendency to lengthen further than the primary wall allows, it is compressed by the elastic primary wall and its parallel texture is buckled to a spiral texture. The slope of the spiral may be to the right or to the left, or in in both directions with many reversals, as is actually observed in cotton hairs.

Preston (42) offers quite another theory of spiral growth, based on the elastic properties of the cell wall. He attempts to find not only a qualitative explanation of the observed features of spiral growth, but to furnish a quantitative theory which permits the prediction of reversals and the calculation of slope angles. He assumes a spiral texture of the primary wall in the growth zone with a flat slope of about 10° under turgor stress. The wall texture is considered to consist of individualized chitin microfibers running more or less parallel, with a certain angular dispersion, so that a mean slope of 10° results. Each microfiber represents a spiral spring. When a weight is suspended on a spring, there is not only an extension ΔL of the spring, but at the same time a rotation $\Delta \Phi$ at the free end of the spring. This rotation is dependent on the torsional rigidity n and the Young's modulus q of the wire of which the spring is made. Spiral growth can be described by expressing the rotation $\Delta \Phi$ in terms of length increase ΔL. Then it is found that this ratio $\Delta \Phi / \Delta L$ is in the first approximation proportional to $1 - 2n/q$.

Young's modulus q depends on main valences in the chitin chain-molecules, whereas the torsional rigidity is a function of the van der Waals forces which bundle the chain-molecules into a microfiber; i.e., n must be roughly 10 times smaller than q, so that the ratio n/q is of the order of $1/10$. Preston calculates from the slope of spiral growth (84.2°) given by Oort (40) $n/q = 0.2$. This figure compares favorably with cellulose where n/q has been found to be 0.155.

As long as the term $(1 - 2n/q)$ is positive, Preston's formula yields a positive rotation, i.e., left-hand spiralling during growth, as is the case in the very beginning and towards the end of the growth of the elongating sporangiophore. If the term $(1 - 2n/q)$ becomes zero, there would be elongation without rotation, and only if this term is negative does a right-hand spiral result. It is not easy to understand how $2n/q$ might turn out > 1. Preston (42) thinks that after the formation of the sporangium, the growth zone temporarily changes the texture of its wall in the sense of a pronounced dispersion of the microfibers. If the angular dispersion of the fibers increases considerably, the elastic properties of the texture are fundamentally altered and no longer comparable with those of individual microfibers; their Young's modulus is lowered, while the torsional rigidity is increased. As these two properties change in opposite directions, there is a possibility that $2n/q > 1$, thus explaining the dextral spiral growth of the sporangiophore when it reassumes elongation after the formation of the sporangium. If the further assumption is made that there is a tendency to readjust the original spiral wall texture by diminishing the dispersion of the microfibers, then the slope of the right-hand growth spiral would gradually become steeper and steeper until the rotation was zero $(2n/q = 1)$ and then reverse to the sinistral growth spiral again.

This theory transfers the enigma of spiral growth to the other unsolved question of how changes in the wall texture are brought about by the cytoplasm. According to preliminary electron micrographs of the *Phycomyces* sporangiophore (25a) the primary wall of this organism has a woven texture

as in primary cellulose walls. Therefore, there is no independence of the individual microfibers as assumed by Preston. In any case, the elastic properties of a single fibril cannot be used to estimate the Young's modulus and torsional rigidity of such a texture. To my mind, the rotation is caused by a circular wandering of the place where the woven texture is loosened. At such a spot the elastic properties of the cell wall are changed to such an extent that it will be difficult to find adequate formulae to describe the behavior of the growth zone correctly.

Dynamics and energetics of cell elongation.—During cell elongation the wall is extended to many times its original length. We may ask how much energy is involved in this extension and find out whether it represents a considerable or only a minor amount of work for a cell. There are two ways of calculating this work (23, 24). The first requires a knowledge of the turgor pressure in the cell, and the second a determination of the tension within the cell wall.

Assuming the turgor pressure T and the change in volume ΔV of the cell are known, the work done A is given by $A = T\Delta V$. The turgor pressure T of a cell is counteracted by the wall pressure W. As long as these two pressures are equal there is equilibrium, and no change of the cell size will occur. The condition necessary to produce cell elongation is $T > W$. Then the cell is no more a static, but a dynamic osmometer which becomes inflated. Whereas T and W have the same value in resting cells, this cannot be the case in growing cells and, therefore, we need a different definition for these two pressures. According to the classical work of Ursprung the wall pressure W is the difference between the osmotic pressure O of the cell sap and the suction tension S of the cell, $W = O - S$. Recently Burström (9) has shown that the turgor pressure must be defined as $T = O - E$, where O is the osmotic pressure of the cell sap and E that of the surrounding medium. Ursprung (56) agrees with this definition. If the system is in osmotic equilibrium, then S equals E, and therefore $T = W$. But if the equilibrium is abolished, e.g., by putting a plasmolized cell $(W = T = O)$ from the plasmolyticum with the osmotic pressure O in pure water $(E = O)$, then the wall pressure W would be zero as long as no water entered the cell, while the turgor pressure would rise suddenly $(T = O)$. Since the osmotic pressure of the diluted cell sap decreases according to the intake of water, the turgor pressure declines also, while the turgidity of the cell increases. This is not the contradiction it seems to be, because the turgidity is actually not due to the turgor pressure, but to the wall pressure, which rises up to $W = O'$ (O' being the osmotic pressure of the diluted cell sap). When equilibrium is reached, the turgor pressure T has been reduced from O to O' and the wall pressure W raised from O to O'.

Burström (8) has found that in the epidermal cells of wheat roots the turgor pressure T remains approximately constant during cell elongation. This object is very appropriate for such measurements because the osmotic concentration of the surrounding nutrient solution is almost zero, so that T always equals O' which value can easily be determined from the concentra-

tion of a solution causing incipient plasmolysis and the shrinking the cell undergoes. The constant turgor pressure being about 3.0 atm., a cylindrical cell with 8 μ radius and an extension ΔL of 437 μ (which means 11.5 times the original length of 38 μ) yields an expansion-work of $A = 0.26$ erg.

Until now it has been believed that the osmotic nonequilibrium in growing cells was caused by an increase of the sap concentration [anatonose (47a)]. But, since there is no apparent rise in turgor pressure, this view must be discarded. On the other hand, it is a problem how the cell can hold its sap concentration during an elongation of some 1,150 per cent without any decrease. As the turgor pressure does not change to any great extent a significant decrease of the wall pressure W must occur to allow of any cell wall extension. Because the wall pressure is due to tensions in the membrane, a consideration of the elastic behavior of the cell walls is necessary.

If we think of a cylindrical cell with radius r and wall thickness d, the longitudinal tension σ on the cell wall is $\sigma = rW/2d$. The transverse tension is twice as great (11) but can be disregarded, as it is counteracted by neighboring cells (24).

When a tissue in which the cells are elongating is plasmolyzed and then put back into the nutrient solution, the tension on the cell wall increases according to Hooke's law in proportion to the elongation $\mathcal{E}$. (The elongation $\mathcal{E} = \Delta L/L$ is the increase in length ΔL, divided by the original length L.) Before the equilibrium $T = W$ is reached the wall seems to stiffen [(13), p. 118], so that σ increases faster than $\mathcal{E}$ (FIG. 1a). If the cell wall reassumes growth, the σ-$\mathcal{E}$-curve bends and becomes more or less parallel to the abscissa, which means that elongation takes place under constant wall tension. If, after a while, we plasmolyze the tissue again, the wall tension decreases following nearly the same curve as for the increase. The wall, however, does not return to zero elongation when the tension is removed. This remaining wall extension BC (FIG. 1a) is considered to represent a plastic deformation (29), while the extension CD is reversible due to the elasticity of the cell wall.

There has been much discussion about the concept of wall plasticity. Because the wall does not become appreciably thinner during cell elongation, Burström (8) denies the presence of plastic extension; he considers every irreversible elongation to be growth, i.e., intussusception of new wall material. This question can be settled by our actual knowledge of the submicroscopic wall texture. As the primary wall represents a woven fabric, its elasticity, as well as its stiffening when considerably extended, are easily understood. But as long as this texture is intact it does not allow of a displacement or shift of the microfibers along each other, which would be a prerequisite for any fluidity or plastic deformation. As we have shown, however, there is such a shift in local regions of a growing primary wall, where the original texture is partly dissolved. At the same time, these loosened areas are readjusted by the formation and interweaving of new microfibers. Thus both phenomena, plasticity and intussusception, occur simultaneously during wall elongation.

The elasticity of the cell wall can be measured, if we consider as a rough approximation that the curves BI and CII in Fig. 1a are straight lines. Then
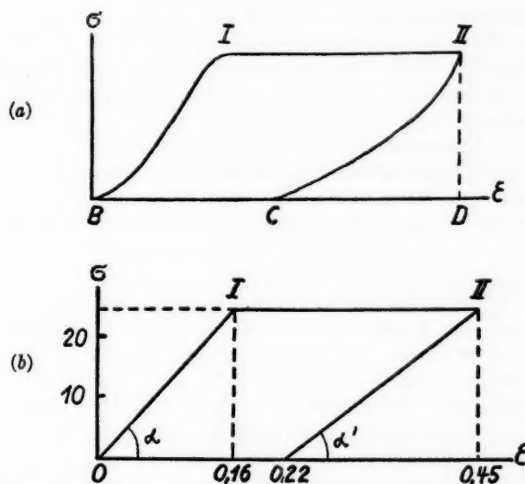
FIG. 1. Tension-elongation diagram of a cell wall (epidermal root cell of wheat). Ordinate: wall tension σ in kg. per sq. cm.; abscissa: elongation $\epsilon = \Delta L/L$. I and II successive states of cell elongation. BC plastic elongation, CD elastic elongation.

    (a) General behavior of the σ-ϵ-curve. The area BI, IICB represents the energy involved.

    (b) Measured example. Young's modulus of the cell wall in the state I is given by tan α and in the state II by tan α'.

Young's modulus q of the wall is given by $q = \sigma/\epsilon = \tan\alpha$ in Fig. 1b. If we make this calculation for numerous time intervals during the growth period of an epidermal root cell of wheat, it is found that the modulus of elasticity decreases considerably when growth starts. It reaches a minimum when extension is at its maximum and becomes larger again when growth ceases (24). The following Young's moduli have been found:

	q
cell prepared to elongate	594 kg. per sq. cm.
cell at maximum expansion	62 kg. per sq. cm.
cell fully elongated	281 kg. per sq. cm.

These figures indicate that growth is accompanied by a fundamental change in the mechanical properties of the wall. It may be incidentally mentioned that these figures compare very unfavorably with those for Young's modulus of cellulose fibers (about 500,000 kg. per sq. cm.), which may be a warning against using the Young's moduli of secondary cell walls when speculating upon growth phenomena of primary walls (42).

    The energy per unit volume involved in the irreversible elongation of the cell wall is represented by the area BI, IICB in Fig. 1a. Considering that during

growth the cell is not plasmolyzed but remains always stretched by the turgor pressure, the area is a rectangle and the work done $A = \sigma\epsilon$. If we calculate this work for each time interval during elongation and add the figures obtained, the energy involved in extending the wall of a wheat root epidermal cell is found to be 0.15 erg. This value compares satisfactorily with the 0.26 erg obtained from the relation $A = T\Delta V$.

The sugar content of such a cell is sufficient to furnish 6.8×10^{-7} cal., whereas 0.26 erg represent only 6.2×10^{-9} cal. This means that the energy used to stretch the cell wall is only 1 per cent of the total respiratory energy available in the cell. Therefore, much more important energy-consuming phenomena must go on in the growing cell.

Metabolism during cell elongation.—Cell elongation is a cytological revolution: a large central vacuole is formed, the cytoplasm is reduced to a thin layer pressed against the cell wall which elongates more than 1,000 per cent, the nucleus is flattened, and, incidentally, differentiation of plastids occurs. These morphological transformations are accompanied by great physiological changes: the cytoplasm of the elongating cell more than doubles in amount (5, 21), every wall substance increases to many times its original weight (23, 59), and the sugar concentration in the cell sap remains constant during a tenfold extension of the vacuole. This means that cell elongation cannot be caused by a mere intake of water, but that there must be considerable translocation of carbohydrates and amino acids, possibly against a concentration gradient, and rapid biosynthesis. These phenomena depend on respiratory energy, so we must expect a considerable oxygen consumption. Wanner (58) found that, in a root, maximum respiration per unit length of the tip occurs in the zone of cell division. But respiration should be calculated in respect to the amount of cytoplasm, because the cell sap and differentiated walls do not respire. Thus if the quotient O_2 consumption/cytoplasmic nitrogen is taken, the intensity of respiration in elongating cells is twice as great as in meristematic cells (33).

The tip growth of root hairs is stopped if the oxygen concentration of the culture medium is lowered to 0.5 mg. O_2 per l. (saturation point 35 mg. O_2 per l.) (33). A few minutes after growth has been interrupted in this way, the root hair bursts at its very tip and the protoplasm is thrown out of the cell. This conspicous plasmoptysis is probably due to the fact that the (enzymatic?) process of wall softening is less sensitive to lack of oxygen than is the synthesis of new microfibers needed for the growth in surface of the softened wall.

The role of auxin in the growth in area of the cell wall is not yet clear. There is no direct action on the cellulose framework. The hypothesis of Ruge (47) that auxin induces a direct swelling of intermicellar wall substances must be discarded (4). As translocation and respiration are of first importance for the growing cell, an influence of auxin on these processes may furnish some explanation. Koningsberger (32) has shown that the permeability to water can be changed by auxin and claims that this is caused by an action on the protoplasmic boundary layer, which is considered to be an

autocomplex-coacervate of lecithin. Whether such an effect can account for all the morphogenetic phenomena caused by auxin is questionable. Much work has been done to find out how auxin stimulates respiration (6, 54, 55). This extensive question is beyond the scope of the present report but has been reviewed excellently by Audus (1). I consider, however, that auxin must interfere with some enzymatic system or systems involved in morphogenesis. If the view expressed above, that the plasticizing of the primary cell wall is due to an enzymatic digestion of microfibers, is correct, then one of the secondary effects of auxin may be an activation of the enzymes in this system.

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POSTHARVEST PHYSIOLOGY AND BIOCHEMISTRY OF FRUITS

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INTRODUCTION

The purpose of this review is to evaluate the present status of the research problems in the field of postharvest physiology of the major fruits used by man as a source of food. The intention of the reviewer is to compare the behavior of the various species for which sufficient experimental results are available, rather than to present a full account of all published studies. It is hoped that this paper will supplement the reviews of several years ago by Smock (1) on the physiology of deciduous fruits in storage and by Miller (2) on the physiology of citrus fruits in storage. Emphasis will be placed here on respiration as the central process common to all fruits, while of the chemical changes, only those will be considered which can be shown to be relevant to physiological behavior. The plan is to utilize different fruit species as subjects of illustration for the several topics. However, the relatively large amount of work on the apple calls for the frequent use of this fruit for illustrative purposes.

METHODOLOGY

The majority of investigators in the field of fruit physiology have limited their respiration measurements to carbon dioxide evolution. The methods used consisted mostly of passing air freed of carbon dioxide at a constant rate over the respiring material and into a suitable alkali containing absorber like a Pettenkofer tube, Truog tower, or through a sintered glass plate. In a Pettenkofer tube absorption of carbon dioxide from the air bubbles is slow and consequently low air flows have to be used. Few workers determined the limiting air rates for their experimental setup, with the result that few of the studies so reported were actually conducted in air. The fruit was subjected instead to an atmosphere much higher in carbon dioxide content than air. The following expression may be used to determine the carbon dioxide concentration in the respiration container:

$$pCO_2 = \frac{R \times W}{F \times 60} \times 100$$

pCO_2 = per cent carbon dioxide in the air stream leaving the respiration container

R = respiration rate in ml. CO_2 per kg. hr.

W = fruit weight in kg.

F = air movement in ml. per min.

Using this formula, one finds a carbon dioxide accumulation of more than

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1 per cent in the case of a sample of apples weighing 5 kg., respiring at the rate of 10 ml. carbon dioxide per kg. hr., and subjected to an air flow of 60 ml. per min. Biale & Shepherd (3) found experimentally a limiting air rate of 150 ml. per min. for samples of lemons which produced only 20 ml. carbon dioxide per hour. Under these conditions the air leaving the respiration container had about 0.2 per cent carbon dioxide.

The respiration methods which depend on the absorption of the carbon dioxide in alkali require the measurement of relatively large quantities of carbon dioxide. In the case of single fruits or with slow respiring material this necessitates tests of several hours' duration. Claypool & Keefer (4) designed a method suitable for tests of short duration. The air leaving the fruit is equilibrated with a dilute solution of sodium bicarbonate, and the resulting pH measured colorimetrically. Since this procedure depends on measuring concentration, accurate flowmeters are essential. A different principle for determining gas exchange in respiration is that of the katharometer described by Stiles & Leach (5). The basis of this method is the thermal conductivity of gases which causes changes in electrical resistance of a wire when heat is removed from the wire. This resistance is calibrated in terms of gas concentration. The katharometer is better adapted for carbon dioxide than for oxygen due to higher thermal conductivity of the former. It has not been used widely because of its complexity and because of the special care required to eliminate several sources of error.

Respiration measurements have been carried out under conditions of $p\,CO_2 = 0$ by the use of the Magness & Diehl (6) method or a modification of it. Carbon dioxide was absorbed in a relatively strong alkali solution and oxygen replenished from a graduated cylinder maintained under constant hydrostatic pressure. This method allows for simultaneous measurements of oxygen absorption and carbon dioxide evolution, but any other gases affecting the respiration process will accumulate in the closed setup. A new development in the field of oxygen measurements is the oxygen analyzer, based on the paramagnetic properties of oxygen. This instrument was introduced recently to this laboratory. It will facilitate respiration studies in the presence of other common gases, notably carbon dioxide, and will supply a continuous record of respiratory activity.

The materials used for fruit respiration studies consisted of composite samples, single fruits, and tissue slices. The composite sample offers the advantage of measuring relatively large quantities of gases. However, because of the induced effect caused by ripe fruit on immature fruit, as described under the heading of plant emanations, particular care has to be exercised in selecting uniform samples. The use of single fruits was thought to remove this objection, but one cannot be certain of uniformity in the ripening process throughout the flesh of the fruit. The use of the tissue slice technique and Warburg manometry might throw some light on this problem. Hackney (7) found no significant difference in respiration rates of tissue slices from various parts of the flesh of the Granny Smith apple. She also

described some problems involved in the use of the tissue slice technique, in particular the marked effects of washing on changes in dry and fresh weight. In the reviewer's laboratory, determinations of oxygen uptake over a period of several hours by tissue slices of the avocado resulted in a straight line relationship between respiration and time. The tissue slice technique was found particularly useful in studying the respiratory mechanisms of the fruit.

THE NORMAL COURSE OF RESPIRATION

The autogenous climacteric.—In the life history of each fruit the following four stages may be distinquished: cell division, cell enlargement, maturation and senescence. The data available for most fruit species point also to a fifth stage at the end of the maturation period, the stage termed by Kidd & West (8) the "climacteric." This phase is of particular interest to the fruit physiologist and also to the student of problems of aging. It marks a transition phase between development and the onset of functional breakdown, between ontogeny and senescence. The "autogenous climacteric" is the marked and sudden rise in respiration prior to senescence which takes place without the influence of external agents, in contrast to the induced climacteric to be discussed below under the heading of ethylene effects. Fig. I illustrates the course of the climacteric for several representative fruits.

The selection of the proper temperature for the observation of the complete climacteric cycle is of paramount importance. If the temperature is too high, the rise in carbon dioxide production might commence before reaching the preclimacteric minimum, while at very low temperatures the difference between the maximum and minimum values might be too small to be considered significant for some species. The climacteric serves as a reference curve, not only for comparisons of quantitative data but also for changes associated with ripening. Transitions from green to yellow in certain varieties of apples, pears, and bananas, or from green to dark brown in some varieties of avocado take place during or immediately following the climacteric. The "eating ripeness" stage of pears coincides with the peak of the climacteric, while in apples, bananas, and avocado it takes place following the peak as a result of chemical changes during the rise. It is also a characteristic feature of the transformations during the climacteric stage that fungal invasion or physiological disorders occur after the peak. Apparently marked, but as yet undisclosed, changes in protoplasmic resistance to disease are associated with this critical stage.

How universal is the occurrence of the climacteric in fruits? The climacteric has been established definitely in several species of fruit belonging to different families, grown in temperate zone regions, tropics, and subtropics, and in fruits of different chemical composition. One feature common to most fruits exhibiting the climacteric is that they contain some reserve substance, like starch or fat, and that they undergo ripening after being harvested horticulturally mature. In contrast to these fruits, oranges, lemons, and grape-

fruit do not undergo any ripening after they are removed from the tree. Their rate of metabolism in air declines slowly without any marked rise in carbon dioxide output. Biale (9) followed the respiratory activity of lemons for seven months at 15°C. and observed a slight rise towards the end of storage life. Alternaria or stem end decay might have been responsible for this behavior. The existing evidence does not support the occurrence of a
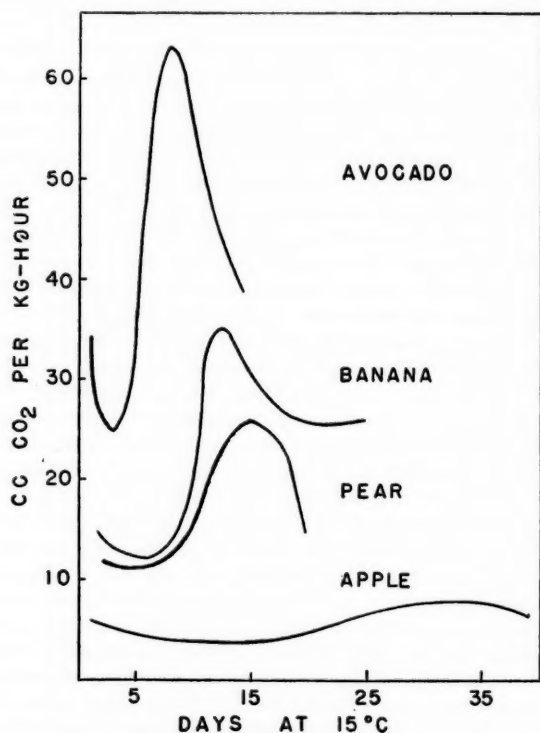
Fig. I.—The course of the climacteric rise in fruit respiration during the postharvest period. (Based on references in text.)

climacteric in citrus in air. On the other hand, Biale & Young (10) did find a characteristic rise in carbon dioxide output by lemons subjected to 34, 68, and 99 per cent oxygen. Unpublished data point to a similar rise in Valencia and Washington navel orang   t oxygen levels higher than air. Apparently a stimulation in respiratory ;   .vity is required to show the climacteric in citrus fruits.

The occurrence of the climacteric on the tree.—Two methods are available for ascertaining whether the typical presenescence peak occurs on the tree: (*a*) placing a complete tree at a constant temperature and enclosing individual attached fruits on this tree in respiration chambers, and (*b*) following the time drift in carbon dioxide production of fruit after gathering. The second method is indirect, but the tree is left in its natural habitat. By the use of method (*a*) Kidd & West (11) concluded that in the apple variety Rome Beauty the climacteric did occur on the tree, but there was great variability between individual fruits in the time of the onset of the rise. Phillips (12) using procedure (*b*) on the McIntosh variety of apples inferred that the peak took place on the tree if the fruit is picked at normal harvest time. Under such conditions he observed better storage quality and greater resistance to carbon dioxide and methyl bromide injury than in early picked samples. Biale (13) showed typical climacteric patterns in Fuerte avocados on early and on late season collections. Here the climacteric did not occur on the tree; neither did the fruit show any tendency towards softening until detached. It is intriguing to know the causes of inhibition of ripening of avocados while attached to the tree.

Respiratory drifts and chemical changes in ontogeny.—The climacteric rise was found by Kidd & West (11) to take place in immature apples as well as in mature fruit. They collected samples of Bramley's Seedling apples at different stages of growth and determined respiration at 12°C. as long as the fruit remained free from fungal attack. The average weight of single fruits varied from 0.65 gm. in May to 245 gm. in September. The corresponding carbon dioxide values were 140 and 6.2 cc. per kg. fresh weight per hour, or 31 and 17 cc. per gm. nitrogen per hr. By choosing nitrogen as a basis, the trends were decidedly different from values based on unit fresh weight. The rate of decline in the early phase was not so marked, while the maximum value throughout development coincided with the climacteric peak. The climacteric occurred in all samples which were collected after the middle of June, that is, after the fruit was about 10 gm. in weight. In the case of samples stored at 10°C. or 12°C. the climacteric tended to occur at about the same time in autumn, while storage at 18°C. accelerated the onset.

Roux (14) followed the respiratory drift of peaches at different stages of development (from 25 to 128 gm. per fruit). He observed early onset of climacteric in young and mature fruit, but delayed in intermediate samples. He suggested, without citing any evidence, that the seasonal changes in the growth of the seed might be responsible for the anomalous response.

The chemical changes associated with the respiratory drift throughout development were described by Kidd (15) for the apple. In the first stage of active cell division proteins formed the chief constituent of the cytoplasmic, nonvacuolar cells. Starch and sugars were present in minute quantities; malic acid synthesis progressed actively. During the stage of cell enlargement vacuoles formed and enlarged until they occupied 80 per cent of the cell

volume. Starch granules appeared in the cytoplasm and sugars mainly in the vacuoles. Total sugar content reached 8 to 9 per cent of fresh weight and consisted of glucose, fructose, and sucrose; malic acid concentration also increased. In the third stage of cell maturation there was a continued slow accumulation of sugars with the production of substances responsible for aroma and flavor, but the acid content declined steadily.

Krotkov (16) found similarities in trends of insoluble phosphorus and respiration during development of McIntosh apples. Hulme (17) observed an increase in net protein content of apples with the climacteric rise; protein synthesis appeared to be correlated with deamidization of asparagine.

In order to study the relation between changes in carbohydrates and respiratory drifts, Kidd (15) harvested apples during the stage of cell enlargement. Thus, observations could be carried on for a long time before the onset of the climacteric rise. Immediately after picking, starch disappeared rapidly. Glucose, the product of starch hydrolysis or phosphorolysis, accumulated, and even fructose and sucrose content increased. As soon as starch was gone, cane sugar declined rapidly until it reached a steady state during cell maturation. Then followed the climacteric with the curious situation that fructose began to disappear for the first time, while sucrose increased along with the rise in respiratory activity. On the basis of these facts Kidd proposed the following theory:

I. glucose⇌active fructose→inactive fructose
  (cytoplasm)   (vacuole)

II. glucose +active fructose⇌sucrose
  (cytoplasm)   (vacuole)

Active fructose is utilized in respiration, while inactive fructose and sucrose accumulate in the vacuoles. Differential permeability between the vacuole and the cytoplasm was postulated as the casue for the accumulation of cane sugar and normal fructose in the vacuole. With the onset of the climacteric when the acidity has fallen to a critical level, a change in permeability enables normal fructose to enter the cytoplasm. In the cytoplasm it is converted to active fructose, the substrate responsible for the rise in respiratory activity. Kidd's theory, while based on many assumptions, suggests an experimental approach to the problem of substrate transformation. According to recent advances in the field of carbohydrate metabolism, active fructose might be fructose-6-phosphate or hexose diphosphate. Determinations of these sugars, coupled with studies of the activity of phosphorylating enzymes, might explain some of the physiological reactions associated with the climacteric.

Internal gas concentration and the climacteric.—The theory for the occurrence of the climacteric based on chemical transformations was presented in the previous section. Wardlaw & co-workers (18, 19) proposed a different explanation as a result of their extensive studies on tropical fruits. They followed the changes in the oxygen and carbon dioxide content inside the fruit

along with determinations of external carbon dioxide. The papaw (*Asimina triloba*) with its large size and hollow interior was particularly suitable for this purpose. During the climacteric stage at 26.5°C. it showed (18) an increase in carbon dioxide evolution from 30 mg. per kg. hr. at the preclimacteric minimum to 120 mg. per kg. hr. at the peak. At the same time the internal carbon dioxide increased and oxygen content dropped sharply. These studies were followed up with greater detail on the banana (19). The banana grown in West Indies or Central America for shipment to Europe and North America is harvested while still green and immature, at a stage described as "three quarters full" when it is only a little more than half grown. During development sugars remain at a very low level, while starch accumulates rapidly. The change from the unripe to the ripe stage is accompanied by sharp rise in respiration, decrease in dry matter, starch hydrolysis, and increase in sugar content, acidity, and glycoside glucose. By inserting a small tube into the fruit and withdrawing a sample of the internal atmosphere, it was found that the climacteric rise in carbon dioxide output was slightly preceded by a sharp rise in internal carbon dioxide and a marked decline in oxygen concentration. In the post-climacteric phase the decline in respiration rates is accompanied by a decrease in carbon dioxide and partial recovery of the oxygen level. During this period the softening tissues offered greater resistance to the movement of gases as indicated by a negative pressure on a manometer. The idea was therefore advanced that internal anaerobic conditions bring about the production of toxic substances and are responsible for senescence and functional breakdown.

The data on internal gas concentration might explain the drop in the respiration curve following the peak, but it does not seem to the reviewer to offer an explanation for the sudden increase in carbon dioxide production during the climacteric rise. Restriction of aeration in ripe fruit was also recorded by Magness & Ballard (20). They found higher carbon dioxide and lower oxygen concentration in pears held at 15.5°C. than in pears held at −1°C. As the fruit ripened at 15.5°C. there was a sharp increase in carbon dioxide and decrease in oxygen content. Consequently the increased respiration rate could not be ascribed to better aeration in the riper fruit.

FACTORS AFFECTING THE CLIMACTERIC RISE

Temperature and life duration.—Fruit respiration is as markedly affected by temperature in the physiological range as the respiration of any other plant tissue. Here two aspects may be distinguished: (*a*) the direct effect of temperature on metabolic activity, and (*b*) the indirect effects caused by low temperature or chilling injury. Both aspects bear a direct relation to the keeping quality and storage life of fruits. The best illustrations for the rôle of the temperature factor are available in the studies on apples, pears, avocados, bananas, and citrus fruits.

Kidd & West (8) observed the climacteric rise in Bramley's Seedling apples at 2.5°C., 10°C., and 22.5°C.; the magnitude for the maximum respiratory activ-

ity was 5 to 6 times as high at 22.5°C as at 2.5°C. It took 25 times as long to reach the climacteric at 2.5°C. as at 22.5°C. They found total carbon dioxide liberated between gathering and end of storage life to be approximately the same for the different temperatures and to correspond to 16 to 20 per cent of the reserve carbohydrates present in the fruit. It is clear, therefore, that it was not lack of "fuel" which caused the respiratory machinery in the apple fruit to break down, but rather a disorganization of the machinery itself.

In a general way the respiration trends in the common varieties of the pear are similar to those in the apples, but the rates of carbon dioxide evolution are much higher, and storage life much shorter. Kidd & West (21) subjected Bon Chretien pears to storage at temperatures ranging from −0.25°C. to 21°C. The ratio of the climacteric maximum to the preclimacteric minimum varied from 3.5 at the high temperatures to 2.5 at the low temperatures. The effect of temperature on duration of the preclimacteric stage was generally small as compared with the effect upon duration of climacteric rise. Magness & Ballard (20) observed marked respiratory rise in the Bartlett variety of pears at 15°C., but none at approximately 0°C. However, upon transfer from storage at 0°C. to 15°C., the peak values obtained were of the same order of magnitude as when placed at the high temperature immediately after picking. Both sugars and acid were higher and the ripened product of finer quality when the fruit was held at 15°C. to 20°C. than at cold storage temperatures. For prolonged keeping it was best to remove the pears from low temperature when the fruit was still firm and green and allow it to ripen at 15°C. to 20°C. When kept at the low temperature too long a chilling injury accompanied by abnormal flavor resulted.

The effects of temperature on the climacteric rise in the avocado were investigated by Biale (22) and by Pratt & Biale (23). The avocado, *Persea americana*, and *Persea drymifolia*, is native to Mexico, Central and South America. Hodgson (24) described the various races of the avocado and methods of cultivation. The Fuerte, the chief variety grown in Southern California, is presumably a cross between the Guatemalan and Mexican races. It is a characteristically high fat, low sugar fruit. Church & Chace (25) found a fat content of 6.97 per cent fresh weight in immature September fruit, and 30.2 per cent in fully mature May samples. The total sugar content for this period changed from 3.06 to 0.13 per cent fresh weight. Protein nitrogen increased for the corresponding collections from 1.50 to 2.32 per cent. Appelman & Noda (26) obtained a sharp increase in oil content of Fuerte avocados between November and March, but no change afterwards. The respiratory activity of early and late gatherings differed mainly by the number of days it took to reach the peak. The response to temperature was very marked. Fruit kept at 5°C. did not exhibit significant variations in carbon dioxide evolution for over two months. Fruits transferred from 5° to 15°C. after three, four, and five weeks showed a sharp increase in respiration to a peak value followed by a post-climacteric drop. On the other hand, fruit transferred after two months passed through a descending curve of car-

bon dioxide production, indicating that the physiological changes associated with the climacteric took place at the low temperature, though measurements of respiratory activity did not lead to this conclusion. This idea is further supported below by a study of production of active emanations.

The significance of the temperature factor in fruit respiration has been further emphasized by the presentation of the values for the temperature coefficient (Q_{10}) for any ten degree range in temperature. Since the rate-time curves differed for different temperatures, comparison had to be made with certain definite reference points. In the avocado (22) the Q_{10} in the range of 5°C. to 15°C. changed from approximately 3.5 in the preclimacteric phase to 7.0 at maximum respiration. For the range of 15° to 25°C. the Q_{10} was 1.81 at the peak. For Gros Michel bananas, Gane (27) obtained a Q_{10} of 2.23 for temperature below 31°C. at the preclimacteric phase. Bananas ripened normally at temperatures between 12.5°C. and 30°C. At higher temperatures the rate of respiration fell rapidly from a high value, the skin did not develop the full yellow color, the pulp became soft and watery, and the fruit was characterized as "boiled." Temperatures below 12.5°C. caused a depression of respiratory activity which at first was reversible, but irreversible effects soon set in and brought about a nearly complete stoppage to respiration. The susceptibility to "chilling" injury appeared to be more pronounced in immature fruit than in material close to the climacteric stage.

Low temperature disorders are also a determining factor for storage conditions of citrus fruits. These physiological disorders include; (a) pitting or formation of sunken areas in the rind with frequent discoloration, (b) brown stain or scald of oranges covering large areas of the rind, (c) red blotch and peteca of lemons, (d) watery breakdown which affects both rind and flesh, and (e) membranous stain causing the darkening or browning of the carpellary walls between segments. The recommendations for storage temperatures depend on whether pathological or physiological disorders cause the greater damage. Under semiarid conditions, such as exist in California and Arizona, there is a tendency to use higher storage temperatures than in semihumid Florida. In California lemons are stored at 13°C. to 15°C., grapefruit at 7.5°C. to 13°C., and oranges at 4°C. to 5°C. Since citrus fruits vary greatly in their vitality, it would be useful to be able to predict storage life. Harvey (28) made an attempt in this direction by enclosing fruit in a jar to which a manometer was attached. At first a negative pressure was produced until the oxygen supply was reduced or exhausted. With continued carbon dioxide production the pressure became positive. A relationship was found between negative pressure and potential storage life, but it was not sufficiently general to be applied in practice. For further discussion of citrus storage problems see Miller (2).

Oxygen, carbon dioxide, and modified atmospheres.—Low temperature disorders, poststorage condition of fruits, and economic factors prompted investigators to search for methods of prolonging storage life in modified atmospheres using temperatures at which chilling injury does not occur. Stud-

ies were directed to the effects of combined changes in the oxygen and carbon dioxide content of the atmosphere, while few workers concentrated on each component separately. Blackman & Parija (29) experimented with oxygen effects on apples. They introduced the concept of the "extinction point of nitrogen respiration," denoting that critical or threshold oxygen concentration value below and above which the rate of carbon dioxide evolution increased. This concept was based on the assumption that a rise in carbon dioxide production at subcritical oxygen concentrations was due to the onset of the fermentative process. Kidd & West (11) found that oxygen concentration influenced the time of the onset of climacteric in apples, and that the magnitude of carbon dioxide evolution was markedly lower at 5 per cent oxygen than at 50 and 100 per cent. They also reported higher threshold oxygen concentrations for alcohol formation in mature apples than in green fruit. Claypool & Allen (30) reported reduced respiration rates of apricots, plums, peaches, pears, and grapes at oxygen levels lower than air. Biale (13) studied effects of oxygen on Fuerte avocados in the range of 0 to 99.4 per cent, and Biale & Young (10) carried out a similar study on lemons. The behavior of lemons was in many respects similar to that of the apple, except that in air no climacteric was observed. In oxygen concentrations higher than air there was a pronounced climacteric, while at lower oxygen levels the rates dropped until the critical oxygen concentration was reached. This critical value varied for different fruit samples and for different storage periods, but generally it was within the range of 0.5 to 5 per cent oxygen at 15°C. The low carbon dioxide readings, coupled with minimum values for the respiratory quotient and with longest storage life, occurred at 5 per cent oxygen. While the rate of chlorophyll disappearance was reduced at lower oxygen tensions, the keeping quality, including resistance to fungi, was best at 5 per cent oxygen.

In contrast with citrus fruits, avocados did not exhibit a critical oxygen concentration. When the oxygen levels were reduced to values below that of air, the rate of carbon dioxide production dropped markedly and the climacteric peak was delayed and suppressed in magnitude. The curve for relative carbon dioxide production at the climacteric peak against oxygen tension had a steep slope in the range of 0 to 5 per cent and decreased progressively until a value of about 35 per cent oxygen was reached, above which there was no more change. The rate of oxygen uptake was observed to follow closely the rate of carbon dioxide output, resulting in respiratory quotients (R.Q.) of the order of 0.81 to 0.96. Generally, there appeared somewhat higher R.Q. values at low oxygen levels, but the differences were not large enough to suggest basic deviations from the normal respiratory process. The rôle of oxygen tension in relation to temperature was also included in this study. At low temperatures (5°C. and 7.5°C.) the effects of oxygen concentration were minimized, with the result that the Q_{10} was markedly different for the several oxygen levels. At 2.5 per cent oxygen the Q_{10} for the preclimacteric minimum in the range of 5°C. to 15°C. had a value of 2, while the corresponding Q_{10} in air was

5. Consequently, greater advantage for storage life at low oxygen levels can be obtained at higher temperatures.

The behavior of the avocado under anaerobic conditions was unique among the fruits studied. Biale (22) found a sharp decline in carbon dioxide output of the Fuerte avocado in an atmosphere of nitrogen. The rate levelled off to a value of about 3 per cent of the values for air. Neither the climacteric nor softening took place under these conditions, and the toxic effect from the anaerobic atmosphere was irreversible. In contrast to the avocado, lemons subjected to nitrogen (10) maintained a high rate of carbon dioxide evolution over a period of three weeks or more. If sugar is the only substrate used at the same rate in both the aerobic and the anaerobic process, and if the fermentation involved is of the alcoholic type, a ratio of carbon dioxide output in nitrogen to carbon dioxide output in air $(N:A) = 0.33$ should be expected. In the case of lemons at 15°C. the $N:A$ ratio was higher than one for over four weeks. When the value became 1, a rapid decline in anaerobic carbon dioxide production started and toxic symptoms became evident. The relationship between aerobic and anaerobic carbon dioxide output in the apple was used by Blackman & Parija (29) to formulate the idea of resynthesis of a cleavage product. The theory of resynthesis of sugars was seriously questioned in the case of muscle and yeast respiration where more experimental results are available than in the apple.

The rôle of carbon dioxide in fruit respiration, despite its obvious importance, is perhaps among the least known subjects. Much of the work was done under variable atmospheres and with methods that depend on high flowmeter accuracy. In the postclimacteric phase at 10°C. Kidd & West (31) found a marked depression of respiratory activity of apples by an atmosphere of 5 and 10 per cent carbon dioxide. In the preclimacteric phase carbon dioxide exerted no marked effect on the magnitude of apple respiration, but it did delay effectively the onset of the climacteric. Thornton (32) observed a marked reduction in oxygen uptake by bananas and strawberries in carbon dioxide atmospheres of 30 per cent or higher. Prolonged exposure to high carbon dioxide caused physiological disorder. Varietal differences in susceptibility to carbon dioxide were ascribed to different internal aeration systems.

The combined effects of reduced oxygen and increased carbon dioxide as background for refrigerated gas storage were summarized by Kidd & West (33). Smock (34)°compared the respiration rates of McIntosh apples in air at 0°C. with respiration in 2 per cent oxygen and 5 per cent carbon dioxide at 4.5°C. The rate of carbon dioxide evolution was one third as fast in controlled atmosphere storage as compared to air storage. Samples removed from the controlled atmosphere condition to air at 19°C. did not respire as fast as the corresponding control fruit. Transfer to air at a high temperature provided evidence for the fact that the climacteric rise was suppressed in the modified atmosphere. Apparently a residual effect persisted which prolonged the period of marketability of controlled atmosphere fruit.

Ethylene and plant emanations.—The rôle of ethylene in plant metabolism generally, and in fruit respiration particularly, offers a fascinating subject for research. We are dealing here with a substance which is physiologically active in minute quantities, but its activity cannot be traced to known mechanisms of substrate degradation. In this review the plan is to discuss ethylene effects on fruit tissue first and to leave problems of ethylene identification and relation to respiration for a separate chapter.

The history of this subject can be traced back to the early years of this century when kerosene stoves were used to color citrus fruits from green to yellow. It was thought that heat was the active agent in this process, but Sievers & True (35) demonstrated conclusively that the incomplete combustion products were responsible for the forced curing. Denny (36) was able to absorb the active gas in bromine and showed that small amounts of pure ethylene were effective. He succeeded in coloring lemons with 0.2 p.p.m. of ethylene in 14 days. Less time was required at higher concentration, though excessively high concentrations of ethylene gave negative results. The presence of oxygen was essential to the process. The coloring of mature green oranges by ethylene was shown by Miller *et al.* (37) to involve an accelerated decomposition of chlorophyll without any significant effect on the carotenoid pigments. Denny (38) obtained an increase of 100 to 200 per cent in carbon dioxide evolution by ethylene-treated lemons at 25°C. The concentration of the gas in air varied from 1 to 1,000 p.p.m. On the other hand, Haller *et al.* (39) did not observe any response in oranges and grapefruit to 10 p.p.m. or lower. Chace & Church (40) showed no effects of 200 p.p.m. concentration of ethylene on reducing sugars, sucrose and acid of juice of lemons, and of Valencia and navel oranges. The reducing sugars sucrose and pentosans, of the peel of these fruits, were not altered by the ethylene treatment. The experimental period was apparently too short to result in greater substrate disappearance of the fruit under ethylene, even though the rate of respiration was consistently higher than in the control. Besides chlorophyll destruction and increased respiration ethylene produces effects such as shedding of "buttons" (calyx and receptacle), greater susceptibility to stem end decay, and general lowering of storage life.

Citrus fruits are not characterized by a climacteric rise of carbon dioxide evolution in air; consequently the ethylene stimulus results in higher rates of respiration. On the other hand, in fruits which have a climacteric, exposure to ethylene does not result in increased rates of respiration for the corresponding stages of the climacteric, but in a shift of the time axis. This was shown clearly by Gane (41) for the banana. If ethylene, even in concentrations as low as 1 p.p.m., was administered in the preclimacteric stage, the onset of the rise in respiratory activity at 15°C. was 12 days ahead of the control. The magnitude of respiration during the preclimacteric minimum or at the peak was not altered by the gas. In the postclimacteric stage ethylene produced no effect. Similarly, the rate of starch-sugar transformation in bananas was not modified by ethylene, but it took place sooner.

Davis & Church (42) studied ethylene effects on two varieties of the Japanese persimmon (*Diospyros kaki*): the astringent Hachiya, and the nonastringent Fuyu. An ethylene treatment of 1,000 p.p.m. was administered for 46 to 52 hr. at room temperature. Since respiration-time curves for the several harvests were not obtained, there is no evidence for the occurrence of the climacteric in persimmons. The increased respiratory activity under ethylene could be explained by earlier onset of the rise in carbon dioxide evolution. The Hachiya was more active physiologically than the Fuyu. In the Hachiya, color, reducing sugars, and weight showed a greater increase, while sucrose showed a greater decrease than in Fuyu. Ethylene stimulated softening, color development, and respiratory activity in both varieties, particularly in the early stages of ripening.

In the species considered thus far ethylene was administered on newly picked fruit prior to storage. Hansen & Hartman (43) working with Bartlett, Bosc, Comice, and Anjou pears compared ethylene effects before and after storage at $-0.5°C$. and at $3°C$. The response to ethylene in the pear, as in the banana, was confined to the stage prior to maximum respiratory activity. The greatest response was obtained with fruits harvested long before the climacteric. Fruit treated after delayed periods of storage showed effective ethylene treatment only during the period of ascending respiratory activity. The varietal differences offered additional support to the observation that ethylene effects are limited to definite stages in the life of the fruit. In the case of the long-lived Anjou pear the fruit responded to ethylene for 12 weeks at $-0.5°C$. On the other hand, the short-lived Bosc variety showed an effect from ethylene for two weeks of storage only. Generally, pears held at $-0.5°C$. responded longer than comparable fruit held at $3°C$. Hansen (44) included in his studies the effects of ethylene on chemical changes in pears. He found increased rate of starch hydrolysis, higher sugar content and more rapid transformation of protopectin to pectin in fruit of early maturity. The reactions in the presence of ethylene were identical to changes that would take place normally in more mature fruit. The length of time during which the individual substances and processes were influenced by ethylene varied greatly, being shortest with sugar and starch, and longest with pectic substances. Respiration and pectins ceased to respond to ethylene at about the same time. In another paper Hansen (45) described definite increases in soluble pectin and decreases in insoluble protopectin due to ethylene in the following fruits: gooseberry, peel of Ponderosa lemon, Elberta peach, Italian prune, hulls of English walnut, Bartlett and Anjou pears. Clearly the softening process induced by ethylene is associated with pectic changes.

The effects of ethylene described in the previous section are closely associated with the rôle of plant emanations in the post-harvest physiology of fruits. A distinction may be made between active emanations which influence respiration and ripening and other volatiles like esters and essential oils of unknown physiological activity. In this review emphasis will be placed on the former.

The observations of investigators on the physiological effects produced by emanations of plant tissue have been largely qualitative in nature. In most instances the responses under study did not lend themselves to satisfactory quantitative evaluations. The first recorded observation in this field was made by Cousins (46) who found that gases from oranges induced premature ripening of bananas. Recent findings with citrus fruits lead one to question whether sound oranges were the cause. It is also possible that bananas in a more advanced state of ripeness caused the ripening of the green fruit. Elmer (47, 48) reopened this field by reporting that apples gave off a gas that inhibited sprouting of potatoes. Huelin (49) found that the emanations from ripening apples and pure ethylene had the same effects on sprouting of potatoes. Gane (50) observed retardation of the germination process in pea seedlings by apple vapors. Epinastic curvatures of potato and tomato leaves were caused by emanations of ripening pears (51) and ripening avocados (23). Isaas (52) demonstrated inhibition in germination of bean seedlings and sprouting of potato tubers by vapors of peaches and plums. Immature fruit as well as other reproductive and vegetative parts of the plant have been found to produce emanations which bring about leaf epinasty (51, 53). It has been definitely established that ripe fruit produces active gas in sufficiently large quantities to induce an immediate rise in the rate of respiration of preclimacteric apples and bananas (41, 54).

Most of the work in this field has been limited to effects produced by gaseous products of higher plants. Little attention has been paid to emanations of microorganisms in relation to activity of higher plant tissue. Gerhardt & Ezell (55) noted a pronounced increase in total volatile substance upon onset of fungal attack, but they did not determine whether these substances influenced any physiological process of the host fruit tissue. Biale (56) and Biale & Shepherd (3) subjected fully mature but dark green lemons to emanations of different storage fungi. They found a sharp increase in carbon dioxide evolution by the sound fruit shortly after exposure to the gaseous products of the common green mold, *Penicillium digitatum*. At 15°C. the maximum increase in respiration was about 100 per cent compared to controls. When the fruit inoculated with the mold was kept at 2.5° and the test lemons at 15°C., no effect was observed. Temperatures higher than 15°C. did not result in a more pronounced response. The active emanation of green mold accelerated, also, the rate of chlorophyll destruction in green fruit. A single moldy lemon was found to bring about these effects in 500 sound lemons, the maximum number used. Unlike the previously reported findings on pears and bananas, lemons responded to the mold emanations throughout their storage life (9). Of several fungi tested (56) the common green mold alone was effective. Miller *et al.* (57) reported leaf epinasty caused by *P. digitatum* and by emanations from normal citrus fruits.

The different responses of plant emanations described here resemble closely the effects of ethylene. In the next chapter the chemical evidence for the presence of ethylene in emanations from ripening fruit will be discussed.

Synthetic growth regulating substances.—The use of growth regulating substances such as α-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic (2,4-D) as sprays to prevent preharvest drop of fruits raised the question of the effects of these substances on respiration and storage life of fruits. Composite samples removed from sprayed trees for the purpose of studying these effects doubtless included fruit which would have dropped prior to normal picking time if it were not for the treatment. Consequently more reliance might be attached to work in which a random lot of harvested fruit was treated after picking.

Mitchell & Marth (58) were among the first to observe acceleration in the ripening of detached apples, pears and bananas by relatively high concentrations of 2,4-D. They determined changes in color and in rate of fruit softening as indicated by a pressure test; no respiration data were reported. Gerhardt & Allmendinger (59) sprayed apples and pears with 10 p.p.m. of NAA. In the case of Delicious apples they found no marked differences in firmness, soluble pectins, and in respiration between the sprayed and control specimens when harvested within 15 days after treatment. When left on the tree for 31 days the sprayed apples showed a somewhat higher rate of carbon dioxide evolution and were more advanced in maturity than the controls. Southwick (60) obtained more marked effects on respiration of Veteran peaches from 2,4-D than from the methyl ester of NAA. Smock & Gross (61) observed higher values for the climacteric peak in McIntosh apples from trees sprayed with 20 p.p.m. of NAA than from control trees. In some cases responses were noticed within three days after spraying, and were more marked at higher temperatures. Hansen (62) studied the effects of 2,4-D on respiration of Bartlett pears in the presence of physiologically active quantities of ethylene. A combination of ethylene and 2,4-D appeared to have a greater effect on respiration and ripening of premature pears than either substance separately. The differences were less marked on mature pears. It seems clear from this study, as well as from the previously reported papers, that whenever growth regulating substances affect ripening they give a higher peak value for carbon dioxide production than the control fruit. Ethylene treatment, on the other hand, results in most cases in a shift of the time axis but not in any increase of the climacteric peak value. The similarities between ethylene and growth regulating substances are based on observations like effects on sprouting and epinasty. However, ethylene is distinctly an abscission-inducing substance, contrasted to abscission-delaying substances like 2,4-D and NAA. Clearly, the available results on the effects of growth-regulating substances are insufficient to make conclusive comparisons with ethylene effects.

PRODUCTION OF ETHYLENE BY FRUITS

Chemical identification of ethylene.—The chemical identification of ethylene among the volatile products of ripening fruits was accomplished in the apple, banana, pear, and avocado. Gane (63) collected the gaseous

products from Worcester Pearmain apples for a period of four weeks in tubes containing bromine at $-65°C$. He obtained 0.85 gm. of oil which, on fractional distillation, yielded 0.65 gm. boiling below 140°C. This oil heated with aniline gave a solid which crystallized from dilute alcohol and had a melting point of 62.5°C. A mixture with a prepared sample of NN′ diphenyl-ethylenediamine of m.p. 62.5°C. also melted at 62.5°C. Gane estimated the total amount of ethylene produced throughout the life of an apple to be about 1 cc.

Niederl *et al.* (64) used a different method for testing the emanations of bananas. Air at the rate of 30 to 50 l. per hr. was passed for seven days over 180 kg. of bananas, when they were at the onset of the climacteric, into a bromine absorption unit. The presence of ethylene would bring about the following reactions.

$$C_2H_4 + Br_2 \rightarrow C_2H_4Br_2$$
$$C_2H_4Br_2 \xrightarrow{\text{alc. KOH}} C_2H_2 + 2HBr$$
$$C_2H_2 + 2AgNO_3 \rightarrow C_2Ag_2 + 2HNO_3$$
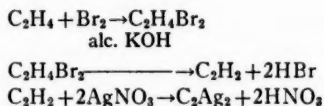
The theoretical silver content of this reaction product is 90.0 per cent; the percentage silver in two runs with bananas was 90.2 and 89.9. If propylene or butylene were given off by the fruit, the percentage of silver would have been much lower than in case of ethylene. This method does not lend itself to quantitative estimation because of the incomplete conversion of ethylene dibromide to acetylene. Allowing for expected errors, Niederl *et al.* estimated an evolution of ethylene of 0.2 to 0.4 ml. per 100 kg. of bananas during a seven-day ripening period.

Pratt *et al.* (65) attempted to identify the active emanation from avocados by the method of Niederl *et al.* (64), but they found it difficult to prevent the rapid loss of bromine. They introduced a modification in the absorption apparatus and used it to collect the emanations of 57 kg. of Fuerte avocados over a period of 139 hr. In view of the high rate of respiration, oxygen instead of air was passed over the fruit. This was justified because it was shown by Biale (12) that the behavior of avocados in pure oxygen was not significantly different from that in air. At the end of the absorption period the fruit was fully ripe, indicating that the collection of the emanations coincided with the climacteric stage. The oil was treated with aniline according to Gane's (63) procedure, and the first derivative obtained was in turn treated with phenylisothiocyanate to yield the second derivative. The following reactions took place:

First derivative

$$CH_2:CH_2 + Br_2 \rightarrow CH_2Br \cdot CH_2Br$$
$$CH_2Br \cdot CH_2Br + 2C_6H_5 \cdot NH_2 \rightarrow C_6H_5 \cdot NH \cdot CH_2 \cdot CH_2 \cdot NH \cdot C_6H_5$$

Second derivative

$$C_6H_5 \cdot NH \cdot CH_2 \cdot CH_2 \cdot NH \cdot C_6H_5 + 2C_6H_5 \cdot N:C:S \rightarrow$$
$$(C_6H_5 \cdot NH \cdot C:S) \cdot N \cdot (C_6H_5) \cdot CH_2 \cdot CH_2 \cdot (C_6H_5) \cdot N \cdot (C_6H_5 \cdot NH \cdot C:S)$$
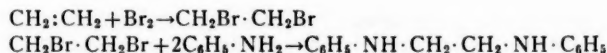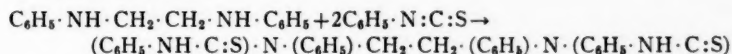
The first and second derivatives after recrystallization gave melting points which agreed closely with the m.p. of the pure chemicals. In later studies in this laboratory this method was modified by employing mercuric perchlorate solution instead of bromine for absorption of ethylene, and releasing ethylene from the complex by chloride ion. It was possible thus to use a simple sintered glass bubbler instead of the more complicated absorption apparatus. The mercuric perchlorate method is being adapted now for quantitative determinations of ethylene.

Hansen & Christensen (66) used a quantitative microbromination method for the determination of ethylene in the emanations from apples and pears. Ethylene is brominated by bromine formed when a slight excess of standard potassium bromate is added to an acid solution of potassium bromide. Upon completion of the bromination potassium iodide is added and the iodine liberated is titrated with standard thiosulphate. The reliability of this method depends on the efficient absorption of other interfering volatiles. Since this method can be used in a closed setup only, the fruit is subjected to a continuous accumulation of active vapors. In order to test for the presence of interfering unsaturated hydrocarbons, the volatiles produced by apples and pears were treated with specific absorbents like 20 per cent mercuric cyanide in 2 N sodium hydroxide for removing acetylene and 87 per cent sulphuric acid for removing propylene and butylene. Ethylene in concentrations less than 20 per cent is not appreciably absorbed by these reagents. Quantitative determinations before and after absorption showed that neither mercuric cyanide nor sulfuric acid (87 per cent) dissolved the volatile vapors of apples and pears. On the other hand, a 20 per cent mercuric nitrate solution in 2 N nitric acid saturated with sodium nitrate which removes ethylene from gas mixtures was found to absorb 92 per cent of Bartlett pear volatiles and 100 per cent of Ortley apple vapors.

It is apparent from this discussion that quantitative methods can be used for ethylene identification, provided suitable precaution is exercised to absorb or test for the presence of unsaturated hydrocarbons and other interfering substances. The quantities of ethylene produced by the various fruits can be obtained directly from the microbromination method (66) or by the assumption that the total amount of oil obtained in absorbing fruit vapors in bromine is ethylene dibromide. With this limitation in mind TABLE I was constructed, using estimates from qualitative methods in the case of Worcester Pearmain apples, avocados, and bananas. The figure for the avocado was substantiated recently by unpublished results from a quantitative method.

Clearly, the production of ethylene varies between different species and

between varieties of the same species. Variations during the ripening period are particularly pronounced and suggest the need to search for relations of ethylene production to respiration and to storage life.

Relation of ethylene production to respiration.—In the earlier studies on the relationship between total volatile substances and respiration, no differentiation was made between active emanations like ethylene and other emanations of unknown role in fruit metabolism. The methods used consisted of

TABLE I

COMPARATIVE ETHYLENE PRODUCTION BY SEVERAL FRUITS

	Variety	Test period	Temp.	Ethylene	Reference
		days	°C.	*ml. per kg. 24 hr.*	
apple	Worcester Pearmain	28		0.12	(63)
	Gravenstein	2	18	0.10	(66)
		9		0.28	
avocado	Fuerte	6	15	0.23	(65)
banana		7	20	0.0005	(64)
pear	Anjou	2	18	0.072	(66)
		14		0.72	
	Bartlett	2	18	5.33	(66)
		6		1.32	
		9		0	

either measuring the quantity of total combustible material or of absorbing the volatiles in concentrated sulfuric acid. Gane (67) found that the amounts of combustible emanation from pears increased rapidly at the climacteric; with apples the tendency was for the more rapidly respiring varieties to produce larger quantities of the volatiles. Kidd *et al.* (68) were able to prove that the increase in volatiles associated with the rise in respiratory activity of Conference pears was not due to an increase in the rates of escape of alcohol and acetaldehyde. Gerhardt & Ezell (69) using the sulfuric acid method found that the climacteric for respiration of Bartlett pears preceded that for total volatiles by a week. They consider acetaldehyde as making up a considerable portion of the volatiles. Walls (70) absorbed the nonethylenic odorous fraction from apples in pure sulphuric acid and ethylene in sulphuric acid containing silver sulphate. Ethylene contributed about two thirds of the total carbon escaping as volatiles.

Studies on total volatiles appear to be of importance if they are followed

up with determinations of some more specific physiologically active emanations. Nelson (71) attacked problems in the physiology of ethylene production by a method which depends on the ability of dilute solution of potassium permanganate to oxidize ethylene to ethylene glycol. Other permanganate reducing substances were removed with sodamide. He found that the rate of ethylene production of McIntosh apple began to increase after the onset of the climacteric rise and reached a maximum several days later than the respiratory maximum. He concluded for the apple and for the banana (72) that ethylene is consumed during the ripening process. It would seem that inflection in the curve of ethylene emanation is insufficient evidence for this contention.

Hansen (73) made a detailed quantitative study of ethylene production in relation to respiration of pears at different temperatures and under different conditions of oxygen tension. Bartlett pears which ripened immediately after picking showed an increase in respiration and in ethylene production about the same time; the maxima in both processes occurred about the same time. During the postclimacteric period, ethylene production and oxygen consumption fell off rapidly, while carbon dioxide evolution showed a much slower rate of decline. The respiratory quotient in fully ripe postclimacteric fruit was 1.4 as compared to 0.8 in initial samples. Apparently qualitative as well as quantitative changes in the respiration process are associated with ripening. A sample of Bartlett pears held for three months at 0°C. showed a high value in ethylene production and in respiration immediately after transferring to 20°C. The more slowly ripening Anjou variety gave a maximum rate of ethylene production of 0.57 to 0.78 ml. per kg. day, as compared to the more active Bartlett variety, which produced 3.25 to 4.48 ml. per kg. day. The relative increases, calculated as maximum rate: initial rate, were much lower for respiration than for ethylene production. These differences prompted Hansen to suggest that the two processes may not be directly related. It would seem, however, that sufficient evidence is available for a relationship between the respiratory mechanism of the climacteric and ethylene production, even though stoichiometric equations do not hold. The quantities involved are of a different order of magnitude, since even at the peak the ethylene formed is less than 0.5 per cent of the carbon dioxide evolved. Hansen supplied evidence for the relation of ethylene evolution to aerobic metabolism. He found that in nitrogen and in hydrogen the output of carbon dioxide was unchanged, while ethylene production dropped sharply. The reaction was found to be reversible.

Hansen also investigated the effect of temperature on respiration and ethylene production. Between 0°C. and 20°C. both processes increased; from 20°C. to 40°C. carbon dioxide evolution continued to increase steadily but the rate of ethylene production declined sharply, reaching a zero value at 40°C. The rate of soluble pectin formation paralleled the ethylene picture. The suggestion was made that at the higher temperatures oxygen concentration might be limiting ethylene output, since it was found that the tissue

oxygen content decreased sharply with increasing temperature. However, experiments with increased oxygen in the atmosphere surrounding the fruit did not result in increased ethylene production at high temperatures. High carbon dioxide in 21 per cent oxygen retarded, but did not inhibit, the formation of ethylene. Addition of ethylene to fruit maintained at high temperatures did not have any effect on ripening, indicating other limiting reactions. The fact that ethylene formation in these studies followed well defined trends shows that it is an integral part of the normal metabolism of maturing fruit.

Pratt & Biale (23) employed the triple response of etiolated pea seedlings (diageotropism, increased growth in thickness, and reduced growth in length) for a study of the relation of the production of an active emanation to respiration in the avocado fruit. The response of the pea seedlings was determined in the range of 0.05 to 10 p.p.m. of ethylene. At 15°C. and 25°C. they found no effect on the peas prior to the onset of the climacteric rise, but as soon as respiration increased, production of the active emanation started, reaching a maximum at the climacteric peak. The behavior at 5°C. was of particular significance. The first response of the pea seedlings was observed some two months after picking. The carbon dioxide values were low and constant during this period. The conclusion was reached that no climacteric took place at 5°C., but when the fruit was subsequently transferred to 15°C. a descending curve of respiration was obtained. Apparently the physiological changes characteristic of the climacteric took place at the low temperature. These changes were demonstrated by the triple response of pea seedlings and not by carbon dioxide evolution.

Relation of ethylene production to storage life.—The recent application of quantitative methods offered the opportunity to relate differences in storage capacity to ethylene production. The results are too few for general conclusions, but some trends might be indicated. Nelson (74) found in six varieties of apples that those with the longest storage life produced least ethylene. Hansen (73) observed a maximum of ethylene production six to seven times greater in the short-lived Bartlett pear variety than in the long-lived Anjou. Hansen (75) studied also ethylene production and respiration in five varieties of apples ranging in season of maturity from midsummer to late fall. The maximum rate of ethylene formation in the July maturing Astrachan was 6.5 times higher than in the October picked Delicious variety. The ethylene determinations on the early varieties were carried out immediately after harvest, while the analyses on the fall varieties took place at different times after storage at 0°C. It is probable that the climacteric in the late maturing varieties had occurred either in storage or on the tree, and consequently they had passed the period of maximum ethylene production.

Fruit Enzymes and Biochemical Mechanisms

The problems discussed in this review suggest a number of questions of biochemical and enzymological nature: (*a*) is the climacteric rise in respira-

tion associated with any change in the respiratory mechanisms of fruits? (*b*) what are the enzymatic activities and what is their relation to physiological behavior? (*c*) does ethylene affect any of the enzymatic activities? (*d*) can ethylene production by the fruit be related to any biochemical mechanism?

At present no satisfactory answers are available for these questions. Many studies were made on a few enzymes, notably phenolases and catalase, but most of the results bear no relation to the problems under discussion here. The physiological rôle of polyphenol oxidase is an open question after the discovery of cytochrome oxidase in the potato. Hackney (76) found increased rates of oxygen uptake by apple tissue slices upon addition of phenolic substances. She argued for the operation of the polyphenolase on the basis of competitive inhibition by resorcinol. The affinity of the enzyme for resorcinol was very much greater than its affinity for catechol. Of particular interest in Hackney's studies is the catalytic action of ascorbic acid. Both Q_{O_2} and Q_{CO_2} were increased by the addition of low concentrations of ascorbic acid to flesh tissue of immature apples. In mature fruit a rise in Q_{O_2}, but not in Q_{CO_2}, took place, and the extra oxygen taken up was no greater than the amount required to oxidize the ascorbic acid. On the basis of these studies Hackney suggested that ascorbic acid may function as substrate for the enzyme at high concentrations and as hydrogen carrier in low concentrations. The terminal oxidase of the apple may consist, therefore, of both polyphenol oxidase and ascorbic acid oxidase. It would be of interest to study the activity of the tissue using ascorbic acid as substrate and catechol in catalytic quantities. In pure apple juice Johnson & Zilva (77) found no enzyme capable of oxidizing L-ascorbic acid directly; oxidation did take place in the presence of catechol.

Hussein (78) observed no polyphenolase activity in orange peel. The respiration of flavedo tissue was inhibited 50 per cent in 0.001 M and 80 per cent in 0.01 M cyanide. The carbon monoxide inhibition was light reversible. He identified cytochrome-*b* spectroscopically. On the basis of these results he concluded that cytochrome oxidase is the terminal oxidase in orange peel respiration. Artsikhovskaya & Rubin (79) reported for the tangerine and lemon a decline in the cyanide sensitive respiration as ripening progressed. Ezell & Gerhardt (80) noticed increased catalase activity with higher state of maturity of apples. In the avocado Appleman (personal communication) found catalase activity increased with the rise in respiration during the climacteric. The high catalase activity in the avocado is of particular interest, because the respiration of avocado tissue slices was cyanide-resistant according to studies conducted in this laboratory. This observation points to the independence of the respiratory system in the avocado from catalase.

The studies on enzymatic activities of fruits as affected by ethylene treatment were concerned mainly with invertase and diastase. Iwanoff (81) reported a threefold increase in invertase activity in the juice of apples treated with 1,000 p.p.m. of ethylene. On the other hand, Regeimbal &

Harvey (82), using the same ethylene concentration, found no appreciable change in total sugars or reducing sugars in the pineapple as a result of treatment. Proteolytic enzyme activity as determined by α-amino nitrogen was not significantly higher in the treated samples. Englis & Zannis (83) did not find any effect of ethylene on the activity of purified diastase and invertase, nor on glucoside hydrolysis. More recently Rakitin (84) observed increased decarboxylation of pyruvic acid by persimmon fruit tissue exposed to ethylene. However, samples in comparable stages of ripeness showed no differences in carboxylase activity.

In discussing the mechanism of ethylene action a number of fruit physiologists cited the coenzyme hypothesis of Lynch (85). This suggestion of ethylene playing the rôle of a prosthetic group is merely based on the observation that both oxygen and ethylene accelerate ripening and respiration of fruits. It is doubtful whether this hypothesis is of any value from the standpoint of suggesting experiments to be carried on. The available results on enzymatic activities of fruits under the influence of ethylene are insufficient and inconclusive. Advancement in this field will doubtless depend on a better understanding of the metabolic cycles in fruit during the normal course of respiration.

Conclusion—Problems of Senescence

The main theme of this review was the physiological changes in fruits which precede, and are associated with, senescence. Attention was focused constantly on the climacteric rise in respiration as the critical stage which separates the stages of development and maturation from the stage of functional breakdown. The climacteric denotes the beginning of the end. Any treatment or condition which delays the onset of the climacteric delays also senescence. Temperature, oxygen, and carbon dioxide content of the surrounding atmosphere, ethylene and active emanations produced by fruit in certain stages of ripening exert a marked influence on the critical respiratory rise. From the standpoint of prolonging storage life the conditions which affect the climacteric must have a quantitative and not a qualitative effect. The changes during senescence proper are not as well known as the changes during the presenescence stage. With some fruits at least it appears that the efficiency of the internal aeration system has decreased. The result is a depletion of oxygen and an accumulation of carbon dioxide. Products of incomplete oxidation are formed and become doubtless one of the causes for the physiological and pathological diseases prevalent in this final stage of senescence.

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RESPIRATION OF HIGHER PLANTS

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Introduction

This review will deal primarily with the literature since James' (1) and Stiles' (2) reviews, which appeared in 1946. Bennet-Clark (3) has reviewed the metabolism of organic acids in plants. The literature on enzymes will be dealt with here only as it is an essential aspect of the problem.

Since there is some disagreement concerning the use of the term "respiration" among plant physiologists (4, 5), we will define the term as used in this review as follows: respiration is the oxidation of organic compounds with molecular oxygen serving as the ultimate electron acceptor; the oxidation may be complete with water and carbon dioxide as the final products, or it may be incomplete, with organic acids as the end products. In contrast, the degradation of carbohydrates into two or more simpler molecules by an oxidation and reduction occurring within the original molecules or its products will be considered as fermentation or glycolysis.

Respiration involves the oxidation of cellular metabolites, with the transfer of the electrons through a series of cellular enzymes (and coenzymes or biocatalysts) to molecular oxygen, resulting in the oxidation of the hydrogen to water and the production of α-keto acids. These acids undergo decarboxylation with the release of carbon dioxide. Thus the energy of the metabolite is released in a stepwise manner from a consecutive series of reactions.

The actual respiratory metabolites are probably rarely hexose sugars, but usually phosphorylated compounds, such as phosphoglyceraldehyde, hydroxy acids, or aldehydes. This involves a common pathway of carbohydrate degradation in fermentation and respiration; in this sense respiration encompasses most of the chemical steps which also occur in fermentation.

The respiratory enzymes thus include the enzymes of phosphorylation, carbohydrate cleavage, the carboxylases, the dehydrogenases, and the oxidases. In addition there are the enzymes (or biocatalysts) transferring electrons from enzyme to enzyme. Hydrogen transport as such need not be assumed, as the liberation of protons to the medium is adequate to explain the observed results.

Some, at least, of the liberated energy is used in cellular work. The most promising mechanism proposed is the coupling of oxidation to phosphorylation and the storage of the energy in a utilizable form in the phosphorylated compounds, such as adenosinetriphosphate.

Respiratory Substrates

The respiratory substrates may be thought of either as the reserve materials, polysaccharides, proteins, and fats, or as the molecules undergoing oxidation by electron loss, such as phosphoglyceraldehyde, pyruvic acid, α-ketoglutaric acid, etc. The reserve carbohydrates are amylopectin (6, 7), amylose, inulin, fructosans (8, 9, 10), hemicelluloses, glycosides (11), and oligosaccharides such as sucrose, raffinose, and stachyose (12 to 15).

Balls & Schwimmer (16) and Schwimmer (17) have succeeded in bringing about the enzymatic dissolution of nongelatinized native starch grains. These authors (18) have obtained α-amylase from barley in crystalline form, and Balls *et al.* (19) have obtained crystalline β-amylase from sweet potatoes. Little is known about the regulation of amylase activity in the cell, but the activation by certain ions has been indicated (16, 17, 20) and the rôle of vitamin C (20) and various inhibitors (21, 22) have been studied. The presence of bound phosphate in native starch is said to diminish amylase activity, and an auxiliary enzyme liberating this phosphate has been reported (23).

The phosphorylase system which catalyzes the equilibrium between starch $+ H_3PO_4$ and glucose-1-phosphate has been shown to occur in potatoes and peas (24, 25, 26), waxy maize (27), sugar beet (28), and a wide variety of plants (29 to 32). The prosthetic groups of plant and animal phosphorylase have been compared (33), as well as the mechanism of action of muscle and potato phosphorylase (34). Arreguin-Lozano & Bonner (35), in a very interesting paper, have studied the action of phosphorylase in potatoes stored at low and medium temperatures, and reached the conclusion that at higher temperatures there is a natural phosphorylase inhibitor produced. The existence of phosphorylases attacking fructosans must be considered very probable, as hydrolytic enzymes for fructosans are largely absent from plants.

In potatoes stored at 0°C. glucose-1-phosphate and fructose-6-phosphate accumulate, while at 25°C., the former completely disappears and the latter is markedly lower in concentration (35). The temperature effect on glucose-6-phosphate is the reverse, for its concentration is maximal at 25°C. The enzyme, phosphoglucomutase, which catalyzes the equilibrium between glucose-1-phosphate and glucose-6-phosphate was demonstrated in pea meal (24, 36); and glucose-1-6-diphosphate has been shown to function catalytically in this transformation (37, 38, 39).

Since the polysaccharides synthesized *in vitro* from glucose-1-phosphate are not identical to native starch, it was suggested that a "branching factor," variously designated as Q-enzyme, cross-linking phosphorylase, iso-phosphorylase or amylose-isomerase, must be present, and satisfactory methods for its isolation have been worked out (40 to 43).

Eyster (44) reports that an inhibitor for the synthesis of starch is present in onions and daffodils, plants that are usually not known to accumulate starch. Extracts of onions inhibited starch synthesis in maize leaves, but the point of attack is unknown. There is evidence that sucrose is a respiratory material just as easily accessible as starch (1), into which substance it is

(biologically) easily transformed (45); the converse reaction is also well-known.

Claims of direct sucrose-formation from starch (46) could not be confirmed (25). Neither has the enzyme sucrose-phosphorylase (47, 48) of *Pseudomonas saccharophila*, establishing an equilibrium between sucrose and free phosphate on one hand, fructose and glucose-1-phosphate on the other, been found in higher plants. In photosynthesis the immediate precursors of sucrose appear to be glucose-1-phosphate and fructose-6-phosphate (49), but the connections between sucrose and respiration, though obvious (49a, 50, 51) remain very obscure.

Weevers (11) has recently demonstrated that in certain willows, salicin functions as a reserve substance. The aglucone, saligenine, arising through salicinase-action, is converted into pyrocatechol. It is tempting, though speculative, to look for a connection here with certain types of oxidative systems such as catechol oxidase. Kursanov *et al.* (52), working on tea leaves, have attempted to link sugars and polyphenols by means of inositol. However, in their system, too, glycosides may be important, since salicin and arbutin synthesized inositol more readily than did sucrose.

It is frequently assumed that the processes of respiration and fermentation are identical up to, and including, the formation of pyruvate. This substance may either be oxidized, perhaps by means of the tricarboxylic acid cycle, or, under anaerobic conditions, it may be decarboxylated and the resulting acetaldehyde reduced to ethyl alcohol. The actual evidence for the general validity of this "common pathway" theory in higher plants is inadequate, e.g., the presence of free pyruvate in onions (52, 52a) can be explained by the finding of Stoll & Seebeck (53) that, in these plants, alliinase decomposes alliine to allicine, ammonia, and pyruvic acid, a reaction that is unlikely to support respiration in intact tissue. In barley, good evidence for the existence of a common pathway was given by James (1); in peas, Meeuse (54) obtained some indication from a study of the change in ratio of fermentation to respiration (F:R) with age. It seems that in the very young pea seedling the activity of the "glycolyzing" enzymes is greater than that of the oxidases, resulting in a high F:R ratio; this falls as the activity of oxidases increases. Phillips Nance (55) has recently pointed out the experimental difficulties connected with the right determination of the F:R ratio, the danger being that carbon dioxide is produced from noncarbohydrate sources, e.g., by severe decarboxylation of organic acids. However, the present writers believe that, in the case of the peas, the observed change is a real one.

As James (1) and others (56 to 59) have pointed out, there is a considerable body of evidence which indicates that mechanisms of carbohydrate degradation are the same in higher plants, yeast, and muscle. Bonner & Wildman (60) have shown that spinach leaf brei can form fructose 1,6-diphosphate and phosphoglyceric acid from added glucose, and at increased levels from glucose and ATP. The occurrence of ATP has been demonstrated in higher plants by Albaum & Ogur (61, 62). Stumpf (63, 64, 64a) has inves-

tigated the occurrence of aldolase, the enzyme splitting fructose-1-6-diphosphate to phosphoglyceraldehyde and dihydroxy acetone phosphate. The enzyme occurs in fleshy fungi, ferns, conifers, and in many families of flowering plants. The aldolase activity is higher in root tips of barley than in the more basal portion, and appears in the cytoplasmic and not the chloroplast fraction of tomato and sugar beet.

PASTEUR EFFECT

Dixon (64) has given the most precise definition of this effect as "the action of oxygen in diminishing carbohydrate destruction, and in suppressing or decreasing the accumulation of the products of anaerobic metabolism." It has generally been assumed that a Pasteur effect could be demonstrated if the ratio of fermentative carbon dioxide: respiratory carbon dioxide is greater than 1/3. (This is the so-called I : N ratio; intramolecular respiration: normal respiration, or better F : R.) This assumption has been challenged by Phillips (65) who points out that it is only justified if one has demonstrated that the fermentation is purely alcoholic and that all of the carbon dioxide is of fermentative origin. She has shown that alcoholic fermentation may be less common in more mature tissues than has been thought, and noncarbohydrate carbon dioxide is produced in anaerobic conditions (Nance, 55). However, whenever the ratio of F : R is greater than 1, there is little doubt that a Pasteur effect occurs (54), and seedlings and some mature plants have a good Pasteur mechanism.

The only unambiguous way, then, to demonstrate the effect, is to study the losses of respiratory substrate in nitrogen and in air. Though the existence of the effect in higher plants has been reported (66), this latter method has been followed only twice, by Fidler (67) for apples and oranges, and by Meeuse (54) for young pea seedlings. The latter author has also criticized Genevois' work (68) on the Pasteur effect in seedlings of sweet pea (Lathyrus). The mechanism of the effect remains obscure. Recently, it has been ascribed to an inhibition of the transphosphorylation between the adenylic system and fructose-6-phosphate (69), an inhibition of the phosphorolysis of glycogen (70), and a slowing down of the splitting of phosphoric acid from phosphoglyceric acid (71). Rudney (72) has studied the mechanism of the inhibition of glycolysis by glyceraldehyde; the 1-isomer inactivates hexokinase. The most important advance is probably the work of Loomis & Lipmann (73), who explained the poisonous action of dinitrophenol on the Pasteur effect by showing that this substance prevents a resynthesis of ATP. Under aerobic conditions there may be little or no inorganic phosphate and adenosinediphosphate in the cell; and if either one regulates glycolysis, a self-regulating system is present. Ascorbic acid (73a) added to slices of sweet potato, carrot, beet, and white potato inhibited anaerobic carbon dioxide production, apparently as an irreversible cell poison. The effect was prevented by hydrogen cyanide, or glutathione or cysteine. Turnips, kohlrabi, and one variety of sweet potato fermented normally in the presence of ascorbate.

MOBILIZATION OF ELECTRONS

The respiratory metabolites produced by the common pathway undergo oxidation by the dehydrogenase coenzyme systems. Surprisingly little direct material has been published on the plant dehydrogenase in the last few years. Earlier studies have established the function of coenzyme I (cozymase) and coenzyme II, and the occurrence of flavines. Meeuse (54) has been able to show that particle suspensions prepared from peas give an increased oxygen uptake when malate or succinate are added with cytochrome-c; and presumably, therefore, contain succinic and malic dehydrogenases. The latter activity was increased upon the addition of coenzyme I but not with ATP.

That certain dehydrogenases are present in specific plants may be inferred from the action of substrates in relieving malonate inhibition. Malonic acid inhibition occurs at pH 4.0 (74), but experiments are difficult since barley root respiration is strongly inhibited at pH 4.0. The inhibition is reversed (75) by pyruvate, succinate, fumarate, α-ketoglutarate, citrate, and cis-aconitate; and therefore one assumes that enzymatic systems are available which act with all these substrates.

Terminal oxidases.—The distribution of cytochrome oxidase and cytochrome-c in higher plants is still a matter of interest. Meeuse (54) has demonstrated not only cytochrome oxidase but also cytochrome-c in pea seedlings. The oxidase and cytochrome-c appear to be bound in the same particles, which may be removed from solution by high speed (16,000 g) centrifugation. Levy & Schade (76) have reached the conclusion that cytochrome oxidase occurs in potato tubers. They find that homogenates have an increased oxygen uptake upon addition of cytochrome-c, and that the respiration of tissue slices is photo-reversibly inhibited by carbon monoxide. The occurrence of the cytochrome oxidase as a distinct enzyme in potato tubers has been established by Goddard & Holden (77). That cytochrome oxidase is widely distributed in plant embryos now seems clear. In addition to earlier reports by others, Bonner (78) has now established it in Avena coleoptiles. It has been thought that this oxidase did not function in mature leaves, but Stenlid (79) has questioned the experimental basis of this conclusion reached by Goddard and co-workers (80, 81, 82) which was based upon the lack of inhibition of respiration of wheat and barley leaves by HCN and NaN$_3$. By lowering the pH to 4.5 Stenlid has succeeded in demonstrating strong azide inhibition. This problem deserves further investigation, but the senior author has already, on other grounds, become skeptical of the nonparticipation of cytochrome oxidase in mature leaf respiration.

Robertson and co-workers (83) believe that cytochrome oxidase is not operative in the basal respiration of the carrot or beet root, as this respiration is not cyanide sensitive. On the other hand, salt respiration is cyanide sensitive.

Smith & Stotz (84) have published a colorimetric method for the determination of cytochrome oxidase, but this has not been applied to plant tissues.

Scarisbrick (84a) has reviewed the literature on the heme compounds in

plants, and reports results on the identification of a new soluble cytochrome-b (b_3) which occurs in leaves and other parts of plants with an α band at 560 mμ, and of unknown function. Further, he reports the presence in leaves, but in no other tissue, of a hemeochromogen extracted in aqueous alcohol that is called cytochrome-f, with an α band at 555 mμ. Cytochrome-f appears to be located primarily in the chloroplasts.

Davison (84b), in a study of the formic dehydrogenase of pea seedlings, reports that cytochrome-c causes an increased oxygen uptake of homogenates in the presence of formate. (There is apparently an error of 10^3 in the reported concentration of cytochrome-c used; the concentrations should be 10^{-6}, not 10^{-9}.) She was unable, in agreement with Meeuse (54), to demonstrate any polyphenol oxidase in peas, but ascorbic acid oxidase was present. In some experiments she obtained an increased oxidation of formate in the presence of added ascorbic acid, but her data are inadequate to justify her conclusion that ascorbic acid oxidase is a terminal oxidase in pea respiration. Hackney (84c) has studied the polyphenol oxidase and ascorbic acid oxidase of apple fruit and shown both enzymes to be present. She draws the conclusion, on a wholly inadequate basis, that both enzymes are terminal oxidases in the apple and that cytochrome oxidase is absent because she failed to isolate cyto-chrome-c. Schade and co-workers (76, 84d) point out that catechol is a cyto-plasmic poison, and that small quantities destroy the endogenous respiration of the potato without inactivating the polyphenol oxidase. They believe that polyphenol oxidase is not a terminal oxidase in the potato. Stenlid (79) is skeptical of the basis for the conclusion of Wildman & Bonner (85) that tryo-sinase is the terminal oxidase of spinach leaves, and Rosenberg & Ducet (183a) found cytochrome oxidase in spinach leaves, an enzyme that Wildman & Bonner reported absent. Rudkin (86) has isolated chlorogenic acid from the sweet potato, and has shown small but consistent increases in oxygen uptake and carbon dioxide production when 1.0 mg. of chlorogenic acid was added to tissue slices of the sweet potato. The tea oxidase participating in the "fermentation" of tea has been established as a tyrosinase by Sreeranga-char (87), and Li & Bonner (88).

Sussman (89) has shown that tyrosinase is widely distributed in plant tissues, since all the species he tested contained tyrosinase. He has also shown that the distinction Onslow made between oxidase (tyrosinase) and non-oxidase plants is not valid. Smith & Stotz (90) have published a quantitative colorimetric method for measuring phenol oxidase in plant materials.

Clagett, Tolbert & Burris (91) have established the presence of an oxi-dase in green leaves of several plants that oxidize gylcolic and lactic acids. The enzyme does not depend upon polyphenols for its activity, nor is it inhibited by azide nor cyanide. The enzyme is absent from several plant embryos or etiolated leaves. Its respiratory rôle is still not established.

The Release of Carbon Dioxide

As Vennesland (92) and others have pointed out, pyruvate occupies a

key position in the metabolism of the cell; it may be converted to alanine (as a possible step to protein synthesis), to fats, to acetate, to acetaldehyde, or to oxaloacetic acid. It may also be oxidized completely through the tricarboxylic acid cycle. Both the conversion of pyruvate to fat and the oxidation are thought to involve a preliminary oxidative decarboxylation, resulting in formation of a 2 carbon-unit which may then undergo certain condensa, tion reactions. The importance of pyruvate may be the reason why attention, until recently, was focused on the α-keto (de)carboxylase attacking this substance (1). The enzyme has now also been found in cereal grains and tomato seedlings (93), in jackbeans (94) and in soybean sprouts (95). Manganese is an effective cofactor, and thiaminepyrophosphate acts as cocarboxylase (95), though the total amount of thiamine varies little (95, 96) during development of soybean sprouts. In ripening fruits, carboxylase activity is reported to be stimulated by ethylene (97). Indirect evidence of pyruvic carboxylase in plants, in the form of observed acetaldehyde or ethyl alcohol formation, is abundant (54, 65). Vennesland & Felsher (98), using direct methods, also found the enzyme widespread. In cucurbit seeds it may play a rôle in germination, but perhaps not in barley grains (99). Schales *et al.* (100) found a glutamic acid decarboxylase, stimulated by pyridoxalphosphate, in a wide variety of plants.

Vennesland and co-workers (101 to 104), in a brilliant series of studies, have demonstrated an oxaloacetate carboxylase (β-carboxylase) in wheat germ, pea seeds, spinach, and in roots of beets, carrot, parsley, and parsnip. The enzyme requires manganese or cobalt for activity. They have established that the reaction is as follows:

$$\text{Oxaloacetate} \rightleftarrows \text{pyruvate} + CO_2.$$
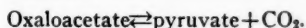
However, the oxaloacetate carboxylase is always accompanied by malic dehydrogenase which is triphosphopyridine nucleotide (TPN) specific, but since a TPN destroying enzyme is present in many plant extracts, a demonstration of the malic enzyme requires protection of the TPN. This was obtained with muscle adenylic acid, ATP, or DPN, but not nicotinamide. The reactions may be written as follows:

$$\text{malate} + TPN \rightleftarrows \text{pyruvate} + CO_2 + TPN - H_2.$$
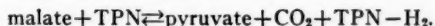
The reaction may be followed on the spectrophotometer at 340 mμ where reduced (but not oxidized) TPN absorbs strongly, and the reversibility of the reaction could be demonstrated. It may be coupled with cytochrome reductase and followed by the reduction of cytochrome-c at 550mμ. Manometrically the reaction was followed by using Warburg's yellow enzyme with sufficient ATP to protect the TPN. It is too early to know whether the malic dehydrogenase and β-carboxylase are activities of a single enzymatic protein.

The fixation of carbon dioxide was shown (101, 105) with radioactive carbon, presumably by a reversal of the oxaloacetate-pyruvate reaction. In the presence of reduced TPN, the reaction proceeds to malic acid, with a small change in free energy.

Synthesis of tricarboxylic acids in plants may proceed by a process involving the addition of carbon dioxide to α-ketoglutarate, similar to the one demonstrated in pig heart and pigeon liver preparations by Ochoa *et al.* (106, 107, 108); this leads to oxalosuccinate and, finally, through the action of isocitric dehydrogenase, to isocitrate (109, 110). The over-all reaction is:

$$\text{isocitrate} + TPN_{ox.} \rightleftharpoons \alpha\text{-ketoglutarate} + CO_2 + TPN_{red.}$$
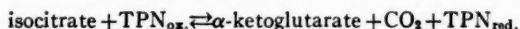
No definite conclusions in regard to the function of this system in plants are drawn by Vennesland, though a rôle in photosynthesis is not considered very likely (92). It is worthy of note that isocitrate (or, when aconitase is present, citrate) can be formed, as long as reduced TPN is generated by some hydrogen donor. The free-energy change involved in the over-all reaction is only small.

The tricarboxylic acid system.—As indicated above, the specific function of this system is the oxidation of pyruvic acid. The exact nature of the 2 carbon-unit thought to be formed through the oxidative decarboxylation of pyruvate is unknown; it may be an acetyl-derivative which exists in combined form only. Its oxidation in the tricarboxylic acid cycle is initiated by condensation with oxaloacetate (111), a process in which coenzyme A, related to pantothenic acid, is involved. According to Stern & Ochoa (112), the condensation-product of acetate (or aceto-acetate) and oxaloacetate, in the case of pigeon-liver preparations fortified by ATP, coenzyme A, and magnesium ions, is citrate. Evidence of the existence of the tricarboxylic acid cycle in plants, then, can be achieved in at least three ways:

(*a*) demonstration of the presence of the substances and enzymes connected with citrate in the way indicated by Krebs *et al.* (113, 114);

(*b*) proof that pyruvic acid is indeed oxidized, preferably in cells or preparations where the normal supply of pyruvate has been blocked by means of poisons;

(*c*) demonstration that interruption of the cycle at a certain point, e.g., by malonate, leads to accumulation of the substance postulated just before the block.

The following data only supplement those already given by Vickery & Pucher in their review (115). Citric acid has been reported in potato tubers (116) and cotton (117, 118). A method for the isolation of optically active isocitric acid from *Bryophyllum* leaves was worked out (119). Aconitase was reported in spinach leaves (85) and rhubarb (120). The belief that oxaloacetic acid as such occurs in the plant kingdom rests exclusively on the claims of Virtanen *et al.* (121 to 125) which could not be confirmed by Wyss, Burris & Wilson (126). Malic dehydrogenase (54, 85) and malic acid (117, 118, 121 to 125) are widespread. Succinic acid has been found in a large variety of plants since Pucher & Vickery (127) developed their method for the determination of this compound in plant tissue. The effect on plant respiration of malonate, known to inhibit succinic dehydrogenase, has been studied by

Turner & Hanly (74). Contradictions as to the effect of malonate that were reported in the past, must be ascribed to the fact that, though the actual inhibitor is the malonic ion, only undissociated malonic acid serves as a cell penetrant, so that a low pH is essential (74, 75). The first to actually postulate a tricarboxylic acid cycle for plants were Bonner & Wildman (60). Further evidence was given by Bonner (128). Laties (75) found that, under certain conditions, addition of pyruvate had a stimulating effect on the respiration of barley root segments in which the normal formation of this keto-compound (through glycolysis) had been blocked by iodoacetate or sodium fluoride. In root segments inhibited by malonate, where formation of succinate was precluded, respiration could be reestablished by glucose, pyruvate, fumarate, citrate, cis-aconitate, α-ketoglutarate, succinate and 1-malate. The negative findings of Henderson & Stauffer (129) could be ascribed to the use of unsatisfactory concentrations and pH's, while the strong inhibitory action of acetate (75) may be explained by the possible competition between this compound and one of the first intermediates in the oxidation of pyruvate, such as acetylphosphate. No explanation is available for Machlis' failure (130) to reestablish respiration in malonate-inhibited barley roots by citrate or the 4-carbon dicarboxylic acids. However, Machlis succeeded in maintaining respiration in the presence of iodoacetic acid by addition of the same substances.

The demonstration of the oxidative formation of succinate from fumarate (or from some closely related dicarboxylic acid) which must be considered one of the strongest criteria for the existence of the cycle, was also achieved by Laties (131) for both spinach leaves and barley roots, in the presence of malonate. Pyruvate and fumarate had an enhancing effect upon the accumulation and displayed an interaction, so that fumarate concentrations which did not elicit a respiratory response by themselves, did so in the presence of pyruvate. Thus it must be concluded that "fumarate, or a fumarate derivative, is involved in the oxidation of pyruvate."

Organic acids.—Despite the reported new findings about the rôle of certain organic acids in the Krebs cycle, it seems likely that, in some cases at least, they must still have some other function; the amounts in which they are present may be very large. This could be interpreted as evidence of a highly specialized type of metabolism. In cotton leaves, up to 20 per cent of the dry weight may be organic acid (118). In succulent plants, marked diurnal variations have been found to occur. Reduced temperature and an increased content of carbon dioxide of the atmosphere increase the amount of organic acid (132); the source of the organic acids seems to be carbohydrate; the evidence at hand does not support the contention that they arise primarily from the oxidative deamination of amino acids. From a series of experiments carried out on *Bryophyllum* (133 to 136) and tobacco (137, 138), Pucher, Vickery et al. came to the conclusion that the various acids active in the Krebs cycle are easily metabolized in these plants, but this evidence alone is insufficient to conclude that such a cycle is operative. As to the indi-

vidual acids, the conversion of malic to citric acid, and vice versa, seems to be a very easy one.

The position of lactic acid in plant respiration is still obscure. A lactate-oxidizing system involving ascorbic acid oxidase (139) was sought in vain in pea seedlings (54). Data about the occurrence of lactic acid in cereal seedlings have been given by Phillips (65).

Oxidation of glycolic acid by barley-leaf suspensions was described by Kolesnikov (140 to 143). Clagett, Tolbert, & Burris (91) found a glycolic acid oxidase in green leaves of many plant orders, but not in etiolated seedlings or embryos: it seems to be specific for 1-α-hydroxymonocarboxylic acids and does not contain easily dissociable cofactors or heavy metal. There is no connection with ascorbic acid, and the rôle of the enzyme in the economy of the plant is not clear.

Oxalic acid in higher plants may be formed from acetic acid via glycolic acid and glyoxylic acid, as is the case in certain fungi (144). In the green plants hydrogen peroxide is usually produced (145) during oxalate oxidation.

A specific dioxymaleic acid oxidase (146) probably does not exist in the plant kingdom (147, 148). However, Kuzin & Doman (149) found a rapid disappearance of infiltrated dihydroxymaleic acid from *Tradescantia* leaves; it was oxidized to diketosuccinic acid. The leaf substances which cause the conversion are extremely unstable. Goddard & Slater, in unpublished work, found that with horse radish slices dioxymaleic acid was rapidly oxidized without catalytic effect on the over-all respiration.

Franke & Schumann (150) reinvestigated aldehyde dehydrogenase (=aldehydrase or aldehyde oxidase, apparently a globulin) from potato. Its rather restricted occurrence in the plant kingdom suggests that other mechanisms for the conversion of acetaldehyde [such as the one reported by Stadtman & Barker (151) which leads to acetylphosphate] may play a rôle in higher plants. The presence of acetic acid itself is very hard to demonstrate, though its presence has been claimed for cambial tissues by Ruhland & Ramshorn (152). Krotkov & Barker (153) have studied the fate of radioactive acetate introduced into tobacco leaves. Though the substance is used, it does not seem likely from their results that acetate is a normal metabolic product in the leaves. It is only in the latter stages of utilization that the bulk of the converted acetate appears as respiratory carbon dioxide. The study of acetylphosphates in higher plants has only just begun (154); Guseva's experiments (155) show that great caution is needed in the interpretation of the results of certain tests for these substances.

RELATION TO DEVELOPMENTAL STAGES

Time-effect.—The idea that there is a differentiation of oxidase mechanisms during the development of the plant (81, 80) has found strong expression in recent work by Mikhlin & Kolesnikov (156). They state that the cytochrome oxidase system is predominant only in early developmental stages, such as embryos; polyphenol oxidase systems are said to be the nor-

mal, terminal oxidases. Artsikhovskaya & Rubin (157) found a marked de-
cline in the HCN-sensitivity of respiration in the late ripening phase of
citrus fruits. On the other hand, Meeuse (54) could not find an important
difference in HCN-sensitivity between young pea seedlings (in which cyto-
chrome-c and cytochrome oxidase were demonstrated) and the green leaflets
of plants about two weeks old.

Quantitative changes in over-all respiratory activity, during plant de-
velopment, have been followed by Merry & Goddard (80) for barley, Meeuse
(54) for peas, and Ruhland & Ramshorn (152) for cambia and root tips of
several plants. The results appear to justify the conclusion that a glycolytic
system is present from the beginning and that an "oxidative" system is
superimposed on this. However, Goddard, Holden & Rosen (158) have found
that young corn root meristem has no fermentative capacity in the area of
cell division, but that fermentation occurs at an appreciable rate in the
elongating cells.

The developmental changes in the amount of certain enzymes and sub-
stances, considered separately, have been followed for a variety of objects
(54, 98, 159 to 163). Useful though these investigations may be, it is un-
likely that they will ever give us the key to the problem of why respiration
increases so rapidly under certain circumstances. A better approach was
followed by Albaum, Novikoff & Ogur (164) who studied both cytochrome
oxidase and succinic dehydrogenase in the chick embryo. For the first two
days, a ratio of 28 was found for the quantities of the two enzymes. After
a gradual change, a constant ratio of 5 was reached after five days. It thus
seems that the low concentration of succinic dehydrogenase may limit
respiration in the very young stages. One of the rare applications of similar
principles in higher plants is found in a beautiful series of investigations on the
spadix of an arum lily, *Sauromatum*, by van Herk (165 to 168). He was able
to show that a hormone, calorigen, originating in the male flowers but diffus-
ing to the "appendix" of the inflorescence, determines the beginning of
respiratory activities of that appendix.

Localization in certain regions.—Sandstedt & Beckord (23a) and Engel
(169) investigated the localization of amylases in cereal grains, Yin & Tung
(31) that of phosphorylase in the epidermis. The latter enzyme is said to
be almost exclusively present in the guard cells. It may play a rôle in the
opening mechanism of the stomata.

Localization within the cells.—Though not much work like that of Claude
et al. (170 to 174) has been carried out on plant cells, the mitochondria
contained in them have been claimed to be the seats of important enzymes
in early investigations. Though unproven, it is not improbable that the
"granular" material isolated by high speed centrifugation from homogenates
of seedlings and certain plant organs by Hill & Bhagvat (175) and others
(54, 176, 177) consists, at least in part, of mitochondria. The following en-
zymes have been shown to be present in this material: cytochrome oxidase
and cytochrome-c (54, 175), succinic dehydrogenase (54, 177), and malic-

dehydrogenase (54). Meeuse (54) found that treatment of the material from peas with an acetate buffer of a pH 4.5 caused the cytochrome-*c* to leave the particles, and based a method for cytochrome-*c* isolation on this observation. However, Engel & Bretschneider (178) could not find a direct relationship between the number of mitochondria and the enzyme content (amylases, proteinase, dipeptidase, and esterase) of various tissues in resting cereal grains.

Plastids are said to be the seats of phosphorylase activity (29, 30, 31). Polyphenol oxidases, though usually occurring in the cytoplasm (85), may also be localized in chloroplasts (88, 179, 180, 181). The oxidase of tea leaves, which according to Sreerangachar (87) is a polyphenol oxidase, is associated largely with water-insoluble particles; this was the main basis for Roberts' belief (183) that tea leaf respiration is largely mediated by a cytochrome oxidase. Sisakyán *et al.* (181, 182) have studied the firmness with which polyphenol oxidase, phosphorylase, peroxidase, and invertase are bound in the plastids. An increase in the osmotic pressure releases the enzymes. The adsorption bond is weak for polyphenol oxidase and phosphorylase, but stronger for the other two enzymes.

Ascorbic acid oxidase, like peroxidase in most cases, seems to be present in solution (54, 139). Wildman & Bonner (85) have given a list of spinach leaf enzymes for which that is also the case. In mushrooms, Belval & Legrand (184) claim that tyrosinase and lactase are associated with the solid structural elements.

Growth and Respiration

Goddard (185) has written a general article on metabolism and plant growth. Several growth inhibitors (emetine, berberine, and colchicine) caused marked inhibition of the growth of barley roots without respiratory inhibition, while the lactone protoanemonin caused marked growth and respiratory inhibition at 10^{-4} *M*. Thimann & Bonner (186) have shown that protoanemonin and coumarin cause marked growth inhibition in the pea curvature test at 10^{-4} and 10^{-3} *M*, respectively, and that the inhibition is prevented by 1,2-dimercaptopropane (British Anti-Lewisite); and therefore the inhibition is probably due to combination with a sulfyhdryl enzyme.

Though there was in the past considerable disagreement about the increased respiration induced by indoleacetic acid (IAA), there seems to be agreement that at levels of 1.0 to 10 mg. per l. it increases respiration, for Berger *et al.* (187) have found increased respiration of 28 to 45 per cent, and Bonner (78) 15 to 25 per cent.

A considerable literature has appeared on the effects of growth regulators on the respiratory rate. Kelly & Avery (188) have studied the action of 2,4-dichlorophenoxyacetic acid on respiration; at a concentration of 1.0 gm. per l. inhibitions of 40 per cent were obtained with pea stems and oat coleoptiles, while at levels between 1 and 100 mg. per l. small, but consistent increases, were obtained with oat coleoptiles. In pea stems increases of 40 per cent were

obtained at 10 and .01 mg. per l. Significantly larger increases occurred with malate and 2,4-D than with either alone. Smith and co-workers (189) observed an increase in oxygen uptake in bindweed rhizomes and roots on treatment with 2,4-D acid; and Smith (190) observed increased oxygen uptake with bean stems. Brown (191) found that 2,4-D at 1,000 p.p.m. caused an 18 to 80 per cent respiratory stimulation of bean seedlings one to four days after treatment. In contrast, Taylor (192) found that 2,4-D at 0.25 to 10 p.p.m. decreased the oxygen consumption of wheat and mustard seedlings within the first hour after application. Hsueh & Lou (193) found that the oxygen uptake of rice and barley seedlings was inhibited about 33 per cent in the first day by 2,4-D at 1,000 p.p.m., while the fermentation remained normal with a marked increase of the R.Q.

Bonner (78, 128) has studied the effects of a series of growth inhibitors and stimulants alone and in combination, on growth and respiration. In addition to IAA, he has found that adenylic acid at 0.1 to 1.0 mg. per l. and arginine at 100 mg. per l. increase both growth and respiration. Canavanine, an arginine analogue, markedly inhibits growth and slightly depresses respiration; its effect is largely abolished by arginine. Presumably, arginine plays a functional rôle in growth and respiration. The increased respiration produced by IAA is abolished by iodoacetic acid, malonate, arsenite, sodium fluoride, canavanine, sodium fluoracetic acid, and dichloranisole. Neither dichloranisole nor canavanine has appreciable effect upon the adenylic induced respiration. Dinitrophenol (DNP) causes increased respiration at low levels (1 to 2 mg. per l.) and marked inhibition at 10 mg. per l. The inhibition is removed by pyruvate, and DNP is unique in blocking growth at levels that stimulate respiration. Space has allowed only a partial presentation of these interesting papers, but Bonner summarizes his results as follows:

Respiration————————→adenylic————————→arginine————————→IAA.
   DNP   acid   step   step
inhibited   step
   step   canavanine
                                      inhibited
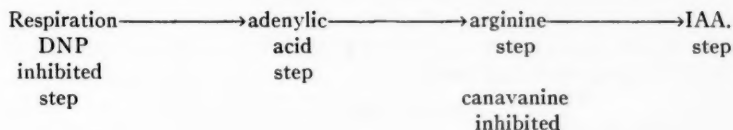
Thimann and his co-workers (194, 195) have been particularly interested in the qualitative changes in metabolism associated with growth; and he suggests that auxin (IAA) causes a qualitative change in metabolism. With oat coleoptile and pea stems he has shown that sulfhydryl-combining substances such as iodoacetic acid, iodoacetamide, arsenite, the organic arsenical mapharsen, p-chloromercuribenzoate, and phenylmercuric salts all inhibit growth, and the growth inhibition may occur at concentrations which cause little or no respiratory inhibition. Malonic and maleic acids protect against iodoacetate without stimulating growth, while malate, succinate, pyruvate, and isocitrate not only protect but bring about a growth stimulation. Growth seems to be correlated not only with sulfhydryl enzymes but also

with organic acid metabolism, and Thimann suggests that IAA may be a switch directing the energy of respiration into the channels of growth.

The correlation of respiratory activity with cellular division and growth has been a field of interest for some years; however, little concrete data exist. Berry & Brock (196) have shown that the first 5 mm. of the onion root tip respires considerably more rapidly than the region from 5 to 10 or 10 to 15 mm. back from the tip. However, mitosis is probably only in a fraction of the 0 to 5 section. Wanner (197) cut wheat root tips into two segments 1.15 mm. long, and the mitosis occurred primarily in the apical piece. She found that on a wet weight basis, more rapid respiration occurred in the mitotic segment. Kopp (197a) found that on a protein nitrogen basis, Wanner's data show a respiratory rate for the basal segment 2.83 times that of the mitotic segment.

Goddard, Holden & Rosen (158) have found that in corn root tips, the most active respiration on a wet weight basis occurs in the first mm., but on a total nitrogen, protein, or ribose nucleic acid basis, the most rapid respiration occurs in the elongating cells 4 to 5 mm. from the tip, and the lowest rate in the cells 1 to 2 mm. from the tip, the very region of maximum mitotic frequency by direct cytological counts. Erickson (198) had already found that in developing lily anthers the respiration was at a minimum when the microsporocytes were in meiosis and the microspores in mitosis. Stern & Kirk (199) have made similar observations on Trillium microspores.

Frey-Wyssling (200) and Goddard (185) have calculated the percentage of the energy released in respiration that is used in growth, and both arrived at an answer of 1 per cent of the total. However, there was an error in Goddard's calculation: for cells doubling in volume per hour the answer should have been 10 per cent; recent unpublished studies from Goddard's laboratory indicate that root cells increase at about 25 per cent in volume per hour, requiring some 4 to 7 per cent of the total energy for osmotic work.

White (201) studied the respiration and fermentation of normal and bacteria-free crown gall tissues; he detected no qualitative differences, but noted slightly lower rates in gall tissues.

SALT RESPIRATION AND ION ABSORPTION

Lundegårdh (202) has reviewed his earlier work showing that the respiration of roots is increased upon the addition of salts; this increased respiration may be calculated as total respiration in salt minus the ground respiration. In two important papers (203), he has added new data and extended his views to include salt transport and bleeding in roots. The ground respiration is essentially cyanide-resistant, while $10^{-4}M$ cyanide abolishes the increased respiration due to the addition of salts and all salt accumulation. These last two papers need a more extended treatment than space allows here.

Robertson et al. (204, 205) have shown that washed carrot slices have a ground respiration which is cyanide-resistant; the addition of potassium chloride causes an increased respiration which is cyanide-sensitive. Beets (206) behave in a nearly similar fashion; while in barley roots (83) no com-

pletely cyanide-resistant ground respiration could be demonstrated. In all cases, however, the extra respiration due to the presence of potassium chloride was cyanide-sensitive.

Robertson & Wilkins (207) have studied the ratio of moles of potassium chloride absorbed: moles of extra oxygen consumed in well washed carrot and beet tissue slices. The extra oxygen consumed is the increase upon the addition of potassium chloride over that in distilled water. The ratio of salt-absorbed oxygen used increases with increasing concentration, and appears to approach four as a limit. Since each molecule of oxygen accepts four electrons and is equivalent to the oxidation of four molecules of ferrocytochrome-c to ferricytochrome, Robertson interprets his experiments as support of Lundegårdh's (202, 203, 208, 209) hypothesis of ion accumulation, in which there is a migration of cytochrome-c in its two valence forms. Though this theory is far from proven, it has received astonishing confirmation in Davies' (210, 211, 212) brilliant work on hydrochloric acid formation in the stomach.

FAT AND PROTEIN OXIDATION

Oxidation of fat in plants.— The metabolism of fatty acids in general has recently been reviewed in a number of articles (213, 214, 215). From work done in various laboratories, it now appears that fat breakdown leads to an acetyl-derivative which may undergo condensation with oxaloacetic acid, resulting in the formation of C_6-tricarboxylic acid. This close connection between fat and carbohydrate metabolism is also evident in plants. Murlin *et al.* (216, 217, 218) found strong evidence for a conversion of oil to carbohydrate in the endosperm of germinating castor beans, the most rapid decrease in fat coinciding with the most rapid gain in sugar. The R.Q. could be accounted for by assuming that 2 out of 6 moles of ricinoleic acid were converted to sucrose and 1 to cellulose, 3 being oxidized. Houget (219), confirming these results in general, claims evidence for the introduction of hydroxyl groups in the ricinoleic acid during its conversion to sucrose. However, to the present authors, a direct transformation without previous breakdown of a fatty acid to a sugar molecule seems unlikely.

Changes in respiratory activity and, concomitantly, in chemical composition of the reserve substances have also been studied in cotton seed (220, 221) and developing tung-fruit (222). The enzymatic oxidation of unsaturated fatty acids by lipoxidases, has recently been considered in a review by Bergström & Holman (223). Lipoxidases seem to be restricted to the plant kingdom but are widespread there (224). At least one has been prepared in crystalline state (225, 226, 227). The effect of various factors on lipoxidase activity and the mechanism of action have been studied (228 to 231). The results indicate that there is no essential difference between enzymatic oxidation and autoxidation of for example, linoleate. The function of lipoxidase in the plant was examined by Holman (232) in a study on germinating soybeans. There is a possibility that the enzyme only initiates the autocatalytic oxidation of linoleic and linolenic acid.

Unfortunately, there is little concrete experimental work on the respiration of amino acids in higher plants. Steward & Street (233), and McKee (234) have recently summarized the modern data about these relations. The mechanism of transamination, which may serve as a link between amino acid and carbohydrate metabolism, has been reviewed by Braunstein (235).

It seems that the enzymes responsible (aminopherases or transaminases) are present in various parts of many plants at different ages (236). Albaum & Cohen (237), from their work on oat seedlings, concluded that there must be a direct correlation between transamination and protein synthesis. Yet, little can be said definitely about the importance of the mechanism in nature; Rautanen (238) concludes that the synthesis of aromatic amino acids appears to be outside its normal scope.

The importance and pivotal position between proteins and carbohydrates of Braunstein's "dicarboxylic acid system" (i.e., aspartic and glutamic acid, with the corresponding ketoacids and a variety of other substances linked with these in some way or other) seems to be well established (176, 233). The essential feature of the connection between respiration and nitrogen metabolism always seems to be the feeding of deaminated residues to the Krebs cycle. From Boswell's (239) and Steward's (233) work on the potato, it appears likely that the nitrogen-free residues of glutamine and glutamic acid, i.e. α-keto-glutaric acid, are drawn into that cycle, while the freed amino groups combine with certain products derived from sugar to form protein. Kretovich & Drozdova (240), studying amino acid oxidation in rye sprouts, found that the dicarboxylic acids, aspartic and glutamic were 3 to 5 times as effective as most of the monocarboxylic acids. The oxidation of glutamic acid was very sensitive to 0.01 M cyanide.

In higher plants, no data are available on the rôle of phosphopyridoxal, which, in pig heart, was found to be the coenzyme for certain transaminations (241).

The behavior of secondary and tertiary amines in the presence of catechol and *Belladonna* catechol oxidase, and the secondary oxidation of amino acids by that enzyme, have recently been studied by James *et al.* (242, 243).

Environmental Factors and Additional Papers

Denny (244) has shown that the retention of carbon dioxide by the tissues may introduce large errors in short term (several hours) respiratory studies if measured by analyzing only for external carbon dioxide. For example, when potato tubers were transferred from 5°C. to 30°C. and the carbon dioxide in the external air and in the tissues was determined, it was found that 81 per cent of the total carbon dioxide produced was retained within the tissue. In contrast, the oxygen consumption may be determined without an error of more than 1 per cent from an analysis of the external gas. He (245) has shown that the internal carbon dioxide of large plant organs is not readily released by evacuation, though he did not acidify the tissue prior to evacuation, so that carbon dioxide retention as bicarbonates

was not excluded. He has introduced a method of carbon dioxide determination, by blending tissue with alkali, and driving off the carbon dioxide after acidification by a stream of air.

Denny (246) has studied the effect of oxygen tensions below those in air on large plant structures such as potato tubers, roots of radish, beet, and turnip, and he found no decreased oxygen uptake at 18 per cent oxygen, about a 5 per cent decrease at 15 per cent oxygen, whilst the decreases at 10 per cent oxygen were small.

It has long been recognized that the respiration of sections of plant organs (carrot root, beet root, potato tuber) have a respiration three to six times as high per unit weight as do the intact organs. Scott (247) has investigated this problem, and showed that it is not due to oxygen diffusion, as the respiration of the organs is not increased by raising the external oxygen pressure. He has investigated the composition of the internal gases of the beet root, by circulating nitrogen in a closed system through a cavity in beet, and showed that in 5 hr. the circulating gas had come to equilibrium with the tissue gases. Within 1 cm. from the outside, the oxygen tension was 13.6 per cent and carbon dioxide 8.2 per cent, while at a depth of 4.5 cm. the oxygen had fallen to 8.6 per cent and carbon dioxide increased to 10 per cent. The low respiration of the whole organ could hardly be due to oxygen lack, but it could be due to increased pressure of carbon dioxide. This could be shown by cutting an internal cylinder the length of a carrot root, and circulating carbon dioxide-free nitrogen through it. This new circulation could supply no extra oxygen, but did lower the internal carbon dioxide tension with an increased respiratory rate. Scott's results, taken as a whole, make a good case for respiratory limitation by high internal carbon dioxide pressure.

Briggs & Robertson (248) have considered the problems involved in the steady state conditions in a sheet of tissue into which oxygen diffuses from both sides and is used by the tissue at a uniform rate, or of the outward diffusion of carbon dioxide which is being uniformly produced. They determined the diffusion constant of carbon dioxide in carrot root disks and obtained values from 1.4 to 2.2×10^{-5} cm.^{2} sec.$^{-1}$ in terms of carbon dioxide concentrations in air in equilibrium with water; this is equal to the water value. They calculated the pressure of carbon dioxide and oxygen at the center of a disk 0.1 cm. thick with a respiratory rate of 8×10^{-5} mg. CO_2 per cm.^{3} per sec. (146 μl. CO_2 per hr. per gm. wet wt.) and found that $pO_2 = 80.5$ mm. Hg and $pCO_2 = 2.3$ mm. Hg.

Berry (249) and Berry & Norris (250) have investigated the effect of external oxygen pressure on three 5 mm. long apical segments of onion roots at various temperatures. From an Arrhenius plot of their data, they calculate a thermal critical increment of the first two 5 mm. segments of 3.9 to 4.1 kcal., indicating a diffusion limited process. They have also calculated the diffusion coefficient of oxygen in the root tissue. These calculations are based on the determination of the external critical pressure required to just give the maximum respiration, and on the assumption that the tissue is homoge-

neous with respect to oxygen diffusion and respiratory rate, and that the respiratory rate of the cells is independent of pO_2; then substituting in the Fenn-Gerard equation, they find that at 15°C. D for the segment 5 to 10 mm. from the apex is 5×10^{-6} cm.^{2} per sec. and at 30°C. 11×10^{-6} cm.^{2} per sec. The respiration of these tissues is high, with Q_{O_2} of 9 to 14.

It is well recognized in animal physiology that after partial or complete anaerobiosis, return to a normal oxygen supply results, for a time, in an increased rate of oxygen utilization, and this is known as "repayment of an oxygen debt." Phillips (65) has shown that barley leaves, after an anaerobic period, repay the debt in part, entirely, or in excess, depending upon the period in nitrogen and the previous history of the leaves. The repayment of an oxygen debt (respiratory overshoot or rebound) has been followed in the apical segments of the onion root by polarigraphic means (251). In the 150 secs. after becoming aerobic, the respiratory rate may increase to 11 times the normal rate, declining in 750 sec. to .33 to .85 the normal. An attempt to deal with the problem on a theoretical ground has been made by Zimmerman (252), but the treatment fails to recognize the physiological data on the concentration of metabolites on the process; and increased rate need not be treated as a stimulant; nor need a physiological stimulant be itself a catalyst.

Warburg and his co-workers (253) feel that by the maintenance of low pressures of carbon dioxide they have shown that the respiration of *Chlorella* is uninfluenced by red light. Further, that the respiratory rate is the same, even at 5 per cent carbon dioxide and at very low pressures. The experimental agreement is excellent.

In a careful study, Duff & Forward (254) have compared the growth stages of wheat leaves with their initial respiratory rates and the time course of respiration during starvation. The young leaves have a high initial respiratory rate, a rapid decline, a second maximum, followed by a gradual decline and death. The older leaves starting out with an initial rate of .3 to .45 of that of the younger, produce less total carbon dioxide, and a respiratory progression during starvation with several maxima and minima and an earlier depletion and death.

Turner (255) has followed the changes in respiratory rate, sugars, organic acids, dry weight, alcohol insoluble residue, total and protein nitrogen, and carbonyl compounds of apples stored at 0°C. over a period of 390 days. Some respiratory fluctuations occur which cannot be accounted for in terms of the sugar concentrations, but a positive correlation with fluctuations in citric acid content may indicate that this acid (or one of its isomers, aconitic or isocitric acids) may participate in the metabolism of carbohydrates. Most of the carbon dioxide produced can be accounted for by loss in sugars or in the alcohol insoluble polysaccharides.

Robertson (256) has shown that most of the respiration of wheat grain (8 to 12 per cent moisture) is due to contaminating insects, and that respiration of the wheat grain is 0.005 μl. CO_2 per hr. per gm. wet wt. or less, the lowest value of respiration recorded.

Denny (257) has proposed modifications of previous methods for the measurement of the respiration of large plant organs. The method depends upon the absorption of the carbon dioxide within the respiration chamber by alkali and subsequent determination of the carbon dioxide by titration. The oxygen consumed is replaced from a reservoir, and the volume of liquid used to displace the oxygen is determined as a measure of oxygen utilization. The microrespirometer described by Gregg (258) which is useful in the range of .005 to 0.1 μl. appears to the authors as the most generally useful, when the experiments require one to work in this range. Laties (259) has described the limitations and use of KOH-KCN mixtures in manometric experiments involving HCN. [cf. Robbie (259a)].

Several general treatments have appeared which will be of considerable interest to the student of plant respiration. Booij & Wolverkamp (260) have published a critical treatment of the concept of the master reaction and its application to a catenary reaction system. This is a highly useful analysis of the uncritical use of temperature coefficients and the assumed rate-limiting step. Dixon (261) has published four lectures in an attractive volume on *Multi-Enzyme Systems*. Here he illustrates the interaction of enzyme systems and the last chapter relating oxidation-reduction potentials of interacting systems to the free energy change will be particularly appreciated. An English edition of Warburg's (262) *Heavy Metal Prosthetic Groups and Enzyme Action* has appeared, and since the German edition was difficult to obtain, and very expensive, this is a welcome edition. The book is written with Warburg's usual verve and partiality, but it is the record of a series of brilliant experimental studies. A new edition of *Respiratory Enzymes* edited by Lardy (263) has appeared, as has a new edition of Sumner & Somers (264) *Chemistry and Methods of Enzymes*. Both of these volumes contain a great deal of new and useful information. A revised edition (265) of the very practical *Manometric Techniques and Tissue Metabolism* has appeared. The sections on the preparations of respiratory substrates and coenzymes are particularly useful.

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THE NITROGENOUS CONSTITUENTS OF PLANTS WITH SPECIAL REFERENCE TO CHROMATOGRAPHIC METHODS

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Evaluation of the literature on the nitrogen compounds of plants presents an ever increasing task. A bibliography of papers published since 1946, when one of us (1) reviewed this same subject, including papers in cognate fields, comprises some 2,000 titles. Many of these papers properly fall within the scope of other recent chapters in the *Annual Reviews of Biochemistry and Microbiology* (2 to 9). Even though many remaining papers are only of indirect interest to the plant physiologist, a large and diffuse literature remains to be evaluated.

General reviews.—Many will wish to refer first to reviews and to primary sources later. After ten years, Nightingale (10) has again reviewed the nitrogenous nutrition of green plants. In this (191 refs.) the point of view is that of the investigator of crop plants who is interested in their over-all nitrogenous nutrition and in the economy of nitrogen compounds in relation to plant behavior. McKee (11) and Street (12) have compiled lengthy summaries each dealing with nitrogenous metabolism in general. The reviews by McKee and Street have attempted much more exhaustive citation of the literature than is possible here. In a further article, Street (13) compiled about 200 references dealing mainly with methods for determining nitrogenous constituents, rather than experimental methods for studying nitrogen metabolism. Since these detailed papers are available, no very useful purpose can be served by still another attempt at comprehensive review; this one, therefore, must be selective, the purpose being to give the reader a preview of the future, rather than a review of the past, by considering topics which seem to indicate the trends to be followed in the future. Since McCalla (14) made the plant proteins a special feature in the last review, this topic will receive minimum attention here; special attention will, however, be given to the soluble nitrogen constituents of plants and to their metabolic rôle.

In a short but lucid article, Pearsall (15) writes in a general vein but has illustrated his thesis by some data. Though protein metabolism of green plants was reviewed by Kretovitch (16) this review was only accessible to us in abstract form. While Lugg (17) states views on proteins and their functions, other general papers deal with proteins from the standpoint of their synthesis and the role of enzymes (18) and also with the response of the

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protein molecule to reagents for specific chemical groups (19) or to protein denaturation. There is, therefore, no lack of general articles with extensive bibliographical treatment.

Nitrogen Compounds in Relation to the Cycle of Growth and Development

A challenging need is to describe the normal growth and development of cells, organs, and of whole plants, in terms of an orderly sequence of events interpretable in terms of their nitrogen compounds. In the 1947 (1) review this was discussed in terms of the varied potentialities of different organs for protein synthesis. Though some of the outstanding problems can be simply stated, their analysis in terms of actual data is possible only to a limited extent.

Growth by cell division.—Growth by cell division implies multiplication by self-duplication; protein-synthesis is commonly held to be a conspicuous feature of cells in this condition, i.e., chiefly cells of the growing regions. In order that one may visualize how a protein may multiply, attention quite naturally turns to the viruses (regarded essentially as crystalline nucleo-proteins which are self-duplicating within certain cells).

The parallelism has frequently been made between viruses and genes, each of which, in different ways, acts upon the cytoplasm. Reviewing virus research in *Science in Progress* (p. 158) Stanley (20) quotes Knight (21) to show that mutant changes in the nature and proportions of amino acids in the virus proteins (tobacco mosaic virus) may modify their effect upon the host and in this way, light may be shed upon the nature of "structural changes which accompany mutation." Following Caspersson (22), some sweeping generalizations could be drawn as follows: (*a*) "reproduction of genes and other self-producing groups could only occur in the presence of polynucleotides"; (*b*) "when we have the right to assume rapid protein-synthesis is going on in a cell, nucleic acids are present"; (*c*) "the nucleus itself is a cell organelle organized for being the main center of the cell for protein formation."

Thus, at the very outset, the student of the nitrogenous constituents of plants is faced with the challenge of unsolved problems. What level of organization in the cell is necessary for protein-synthesis?[3] How far may protein-synthesis be regarded as the summation of steps, each of which emerged separately in evolution, leading to the self-duplicating nucleo-proteins which would be regarded, by Caspersson, as the templates of synthesis? Beadle (23) also referring to Horowitz (24) has attempted to visualize how the chains of stepwise reactions, each gene controlled, could have been built up.

Evolution and protein-synthesis.—The central idea of Beadle's, and of other similar discussions, is open to serious question. The tendency is to deduce from what is known about genes and virus nucleo-proteins something of the nature of primitive self-duplicating units and the circumstances of

[3] See later section on synthesis in tissue homogenates (p. 259).

their origin. Inasmuch as genes and viruses are only known in an already highly organized milieu, i.e., as the very end products of the evolutionary process, there seems no reason to believe that they are the modern counterparts of units which originated before life existed in its present forms. To us it seems that the remarkable evidence of biochemical genetics, largely the outcome of work on *Neurospora* mutants, is being extended in two quite unacceptable directions.

It has already been pointed out [cf. (1), p. 477] that one cannot prove the sequence of reactions by which an autotrophic organism or a cell makes protein, by furnishing evidence from mutants that have become heterotrophic for a given substance. Biochemical genetics can only demonstrate that a given mutant must have a given intermediate; it cannot prove that the normal autotrophic cell, which does not require that substance to be supplied, necessarily uses it as an intermediate. And so far as we can see, the evidence of biochemical genetics does not and probably cannot furnish the proof of the steps by which the autotrophic plant cell makes protein.

Similarly the evolutionary argument does not seem to us to hold. The genetical evidence is now interpreted to mean that protein or nucleo-protein synthesis became possible following the development of all the separate mechanisms for the genic control of each requisite step. This again overemphasizes the evidence derived from heterotrophic mutants and seems to lose sight of the obvious fact that life must in its genesis have been autotrophic, capable of existing in an inorganic world. One should recognize the more autotrophic type of nutrition as being, by this token, more primitive than the heterotrophic. While organisms have become morphologically more advanced, they have become metabolically less self-sufficient.

The relevance of this discussion is that the amino acid hypothesis of protein-synthesis, with its corollaries drawn from biochemical genetics, should not be regarded as established merely because it is now being extended in these intellectually stimulating directions.

Cell growth and development.—Cell growth by elongation, in contrast to cell division, presupposes increase in substance but places different biochemical demands upon the centers of synthesis. In turn, maturity, senescence, and death, (or the normal loss of protoplasm as part of the process of tissue differentiation) foster breakdown instead of synthesis and the release of nitrogen compounds which become again available for synthesis in other parts of the plant body. In the rest period and in dormancy—as in diapause in insects—protoplasmic synthesis and activity is arrested, not by any obvious external and environmental factors, but by mechanisms inherent in the cells. Thus far, the problem is stated without regard to the additional complications of the entire life cycle, which comprise the almost universal inability of the individual gametes to grow and synthesize indefinitely, and the equally remarkable stimulus to synthesis after fertilization occurs. Likewise meiosis and spore formation present different problems, for here the morphological events are accompanied, if they are not caused, by the interruption

in the orderly sequence in which protoplasmic synthesis proceeds *pari passu* with cell and nuclear division. At meiosis, there occur divisions without apparent synthesis and nuclei are formed with half the previous allotment of chromosomes and, presumably, half the kinds if not half the actual amount, of nucleo-protein materials or groups. These are the sort of problems inherent in biochemical ontogeny of nitrogen compounds. How are they being attacked?

Different systems may be used. Growing cultures of algae furnish material in which the cells pass collectively through the phases of cell division, cell enlargement, and maturity. Along the axis of a single root biochemical changes may be traced as cells pass from active cell divisions to maturity. In a succession of leaves born on the same axis the changes with age and development may be traced. Isolated tissues, or even embryos growing in sterile culture, serve as material in which to investigate the characteristics of actively growing cells. In such materials as anthers, the consequences of meiosis for nitrogen metabolism may be investigated.

Pearsall (15) points out that as the leaf grows, and the total-nitrogen content increases, the relative content of protein (protein-nitrogen as percentage of total-nitrogen) decreases. With the enlargement of the leaf, however, the nature of the protein-nitrogen synthesized changes progressively from a protein relatively rich in the basic nitrogen fractions characteristic of the nucleo-proteins, to proteins relatively poor in these fractions. The decline in synthesis of basic-nitrogen rich proteins is held to parallel the decline in cell division as the leaves develop.

Reanalyzing and recapitulating his views on the effect of age, development, flowering, light and darkness, nitrate and sugar supply [cf. (1)] on protein-synthesis, Pearsall remarks that "a knowledge of the physiology of protein-synthesis and degradation will provide an important means of integrating many phenomena of plant metabolism," and, it may be added, development.

The degree of importance of the period of elongation in the metabolism of nitrogen compounds is more often inferred than established. Frey-Wyssling & Blank (25) state that the elongation of the coleoptile of *Zea*, in which cell division plays a minor rôle, is accompanied by a considerable protein-synthesis since the cells are said to increase their protein to 9.5 times the original amount. Frey-Wyssling (26), quoting data from Frey-Wyssling & Blank, shows that cells which are elongating may increase their protein-nitrogen very greatly and rapidly by synthesis from soluble-nitrogen compounds, even though the protein-nitrogen relative to the fresh or dry weight is on the decline. Frey-Wyssling also calculated that the fraction of the respiratory energy devoted to actual cell extension is very small. For absorption of specific salts, this was shown by Steward (27, p. 440) some time ago, and now Frey-Wyssling regards the almost explosive enlargement of cells, not as a passive water intake, but as a process which is concerned with protoplasmic synthesis. The review by Steward & Street pointed to the prominent

fact that it is cells capable of synthesis which can take the processes of salt intake in their stride.

Goddard (28) anticipates that new chemical techniques for measuring nucleo-proteins (29) will make it possible to trace out the synthesis of protein, not only through the normal ontogeny of living cells, as e.g., in roots, but also through the cycle of meiosis and sporogenesis by using selected material as e.g., anthers, following Erikson's technique (30). Such a picture of the growth and development of plant cells in terms of the synthesis of their nitrogen compounds is urgently needed.

Tissue cultures of cells isolated from the plant body and endowed with the ability for indefinite proliferation, present favorable material to study the relation between the nitrogenous metabolites and the ability to divide. Tissue cultures are of different kinds: (a) cells which are originally stimulated to division by pathogens and then rendered sterile, but which have retained the capacity for maintained synthesis and division; (b) tissues which are of cambial origin and which may be stimulated into growth by auxins (indole-acetic acid, etc.) and (c) cells which are otherwise mature and which only recover the ability for rapid growth and proliferation under the influence of specific growth substances (31, 32).

A striking example of the latter is to be found in the mature secondary phloem of the carrot root, which responds to an active and specific principle in coconut milk by active and very rapid growth (33, 34). A specific effect of the coconut milk factor is to promote protein-synthesis. The initial cells have the bulk of their nitrogen (60 per cent) in the alcohol soluble form and they show only a restricted ability to synthesize protein from this or from nitrate which may be absorbed. In contrast, however, stand the cells treated with the coconut milk factor, for these absorb nitrogen from the external solution and convert it into protein. Clearly the factor in the liquid endosperm (coconut milk), which is also found in morphologically comparable situations, such as the immature fruit of *Zea* (corn in the milk stage), not only stimulates the development of the coconut embryo, but also behaves as a protein stimulating substance toward mature carrot phloem and also to certain other adult tissues. [This effect is quite apart from the supply of nitrogen to the embryo from the endosperm. Yemm (34a) now argues that, judging from the large amount of glutamine in the protein of the endosperm of barley, this is the form in which nitrogen is furnished to the growing embryo.] These observations suggest some striking possibilities in the relation of protein-synthesis to growth and development. Does the embryo of flowering plants receive factors from the nucellus and endosperm analogous to the Animal Protein Factor (A.P.F.) of the animal body, which stimulate the cells to protein synthesis? Is this activity controlled during subsequent development, as cells and organs differentiate and mature, by the presence of other regulatory substances (inhibitors) or is it that the activity itself disappears? Thus, the causal connection between the random proliferation to form tumor-like growth in plants and the protein-synthesis which this growth

entails seems now to be mediated by one or more specific, water soluble and heat stable substances.

PAPER CHROMATOGRAPHY IN THE IDENTIFICATION OF NITROGEN COMPOUNDS

In the review by Steward & Street (1), the importance of partition paper chromatography was anticipated in a footnote. Even in the recent review of experimental methods by Street (13) these new tools for the investigation of nitrogen constituents command only a very brief mention. Since these techniques represent the most important recent development in the investigation of nitrogen compounds, the subject will now be discussed in detail.

Partition chromatography: general considerations.—The theory of partition chromatography, which utilized the property of partition between two solvents, where one of the solvents is held stationary on an inert material, has been discussed by Martin (35). In 1944, Consden, Gordon & Martin (36) published the first paper on paper partition chromatography for separation of amino acids. They established the basic principles of paper chromatography which with many modifications have also been utilized in separating many types of compounds other than amino acids [for general articles *see* (37), (38)].

Chromatography on paper is applicable to small quantities of material and by chromatographing first with one solvent and then another in a direction at right angles, two dimensional separations can be accomplished. Paper chromatography has been used mainly for qualitative separations and identification but many techniques now exist which purport to make the method quantitative. Column partition chromatography gives less complete separations because it is one-dimensional, but it has the advantage in that it is adaptable to larger quantities of materials and is therefore more useful for isolation purposes. The paper "chromatopile" of Mitchell & Haskins (204) is also applicable here. Haugaard & Kroner (39) have introduced an interesting technique which gives much better separations in a one-dimensional chromatogram on a filter paper sheet. The phenol solvent is saturated with a buffer at pH 6.2. Two metal strips are threaded down the side of the filter paper to act as electrodes. Electrophoresis takes place simultaneously with the chromatography, thus separating acidic, basic, and neutral amino acids.

Paper chromatography of amino compounds.—Consden, Gordon & Martin (36) utilized stoneware drain pipes for one-dimensional work and gas-tight cabinets (30 × 30 × 5 inches) for two-dimensional work. In two-dimensional work, the filter paper sheet is folded along one side and the fold is placed in a glass trough with the paper hanging down. The moving solvent is put into the trough and allowed to run down the hanging paper. After removing the first solvent, this procedure is repeated in a direction at right angles. Enameled metal (40), stainless steel (41) and ceramic troughs[4] have been used. Williams & Kirby (42) introduced the capillary ascent method which has

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[4] Private communication from Botany Department, Cornell University.

some advantages in simplicity of apparatus and consistency of results but obvious disadvantages due to slowness of movement, limited length of travel and to the degree of separation which is obtainable. Longenecker (43) and Winsten (44) have described special apparatus for the separation of small amounts of materials. Wolfson *et al.* (45) have described an apparatus which is convenient for the preparation of large sized two-dimensional chromatograms by the capillary ascent method. By attaching a thick pad of cellulose tissue at the foot of descending chromatograms (60 ×60 cm.) Miettinen & Virtanen (46) achieved advantages which would otherwise have required very long chromatograms with a long time to develop (two to three weeks in tert. amyl alcohol): they allow the fastest moving constituent to reach the pad. Rockland & Dunn (47) have used paper strips in test tubes to separate amino acids present in less than microgram quantities.

The solvents recommended by Consden *et al.* (36) are the most effective for separation of amino acids. A weakly acidic solvent (phenol) was used in one direction and a weakly basic solvent "Collidine" in the other. For unidirectional work phenol is most widely used. It is now known that the collidine first used contained lutidine, and a mixture of 2,4,6-collidine and 2,4-lutidine in some fixed ratio between 1:1 and 1:3 is now recommended. Solve; ts related to phenol, e.g. the cresols (48) and solvents related to collidine and lutidine, e.g. pyridine (49) are also used. Alcohols are also widely used for separating amino acids: not only the primary alcohols such as propyl, isopropyl, butyl, and benzyl alcohols etc. (50) and furfuryl alcohol, (51) but also tertiary alcohols such as tertiary amyl alcohol which is specially recommended for its resolving power (46). The alcohols are preferable to collidine because they are less prone to decompose cystine (46, 52). Borsook *et al.* (53) employed carboxylic acids (butyric acid) in the separation of α-amino adipic acid from glutamic acid.

Consden, Gordon & Martin (36) chromatographed with solvents saturated with water. This presents difficulties if the temperature changes during chromatography and was recognized by Bull, Hahn & Baptist (54) who utilize solvents which are not saturated with water. In general, solvents which are partially miscible with water are preferred. However, Bentley & Whitehead (51) have obtained satisfactory separations with furfuryl alcohol and tetrahydrofurfuryl alcohol which are completely miscible with water.

Water is not the only solvent that has been utilized for the stationary phase. Williams & Kirby (42) employed a saturated sodium chloride solution. Zaffaroni *et al.* (55) used propylene glycol or formamide for the stationary phase and benzene as the mobile solvent. To determine the proper solvent one may test a number of solvents very easily by using the capillary ascent technique in test tubes. Solvents should be pure to ensure reproducibility of results and also to avoid oxidations that may be catalyzed by impurities.

Consden *et al.* (36) noticed oxidation of the phenol, attributed to the presence of heavy metals (particularly copper) in the paper. They reduced this oxidation by using metal complexing agents. Draper & Pollard (56) con-

sider that most of the heavy metal contamination is in the phenol, which they therefore distilled over aluminum and sodium carbonate under reduced pressure. The oxidation of phenol also tends to be increased by the slight amount of ammonia which Consden *et al.* include in the cabinet in order to confine some of the amino acids to a small area. In this laboratory, both ammonia and cyanide have been omitted from the procedure to avoid the risk that traces of ninhydrin-reacting substances might be formed thereby and to avoid the oxidation of the phenol. This alters the location of some (mainly basic) amino compounds on the paper. The filter paper used by us is usually Whatman No. 1 following Consden *et al.*, who also used Whatman No. 42 satisfactorily. Whatman No. 4 has also been used, as has Munktells O B (49) and Schleicher and Schuell No. 507 (54). In this laboratory a number of papers have been tried, but none has been found superior to Whatman No. 1. Wynn (205) has found that Whatman No. 1 contains some polypeptides: this has a bearing upon the isolation of pure products from the paper, but it does not invalidate paper chromatography as commonly practiced. Kowkabany & Cassidy (206) have examined 75 types of paper in relation to amino acid chromatography and they make several recommendations.

Chromatography is usually carried out at, or near, room temperature. The actual temperature is not critical, but it is important that the temperature should not fluctuate during chromatography if the solvents used are saturated, because one solvent may separate out from the other. Consden *et al.* have shown that the R_F values for amino acids are changed by temperature because, using saturated solvents, the amount of water in the solvents changes with temperature (R_F being the ratio of the movement of the substance to the travel of the solvent front). The use of unsaturated solvents would minimize this effect for limited temperature changes.

Many substances interfere with the paper partition chromatography of amino acids. Complete removal of salts initially present in the paper, which gave it an alkaline reaction, influences the chromatograms of glumatic and aspartic acids by accentuating their tendency to "streak." Salts in high concentration tend to take water from the solvent and "water log" the paper so that the amino acids do not move. To avoid this, Williams & Kirby (42) equilibrated their phenol with a saturated sodium chloride solution. Consden *et al.* (57) point out that in the presence of smaller amounts of salts one gets a separation of cations and anions, and that a yellow spot often observable in the glycine region may be due to the effect on the ninhydrin of a local excess of cation over anion. [Some yellow areas (Nos. 25, 26 & 27) in the early chromatograms of Dent, Stepka & Steward (81) are no longer obtained by current procedure in which pure solvents are used.] Consden *et al.* found that in this region they could obtain better color development if the ninhydrin was somewhat acid. They have also described an electrodialysis procedure which separates amino acids from salts before chromatography (58). In our experience, high concentrations of sugar will distort the chromatograms as will probably any water-soluble substance present in relatively large concentration.

Several methods of detecting amino acids have been employed. Consden *et al.* (36) used 0.1 per cent solution of ninhydrin in *n*-butanol which was sprayed on the paper. Ninhydrin forms a color with compounds having a free primary amino group (not just α-amino acids as is often stated) to form a blue color. Amide groups do not form a color. Proline and hydroxyproline, with imino groups, form a yellow-brown or reddish product which is different from that formed by primary amino groups (59). The color develops slowly at room temperature and rapidly above 50° to 60°C. In this laboratory, ethyl alcohol has been used successfully as the ninhydrin solvent. The ninhydrin reaction has the merit of sensitivity. The limits of detection of many amino compounds have been determined by Pratt & Auclair (52) and their results are in accord with the view that ninhydrin is not a sensitive reagent for histidine and peptides. The general experience is that cystine-cysteine react poorly with ninhydrin. Although the colored product of the reaction of ninhydrin with all primary amino groups should be the same (60), there are, however, observable differences in color on the paper, probably due to secondary reactions which are not commonly controlled. There is evidence that the marked difference in apparent sensitivity of the test for different amino acids is due to unsuitable conditions for color development (unpublished data). Other general reagents for the detection of amino acids that have been tried are the Folin reaction (61) and fluorescence (62). Martin & Mittelman (61) utilized the Folin reaction which is less sensitive than the ninhydrin test. Many amino acids fluoresce on the paper and the amount of fluorescence is increased by heating (63); this test is, however, not as sensitive as the ninhydrin test, and other fluorescent materials interfere.

Another general means of detecting amino acids on the paper has been by radioactivity (64 to 68). Keston *et al.* (64) reacted amino acids with a compound of radioactive iodine. The resulting compound was then chromatographed and its position is detected by a Geiger counter; a special technique for measuring radioactivity on such papers has been described (67). Fink & Fink (65) and Tishkoff *et al.* (68) have fed radioactive iodine to animals and then followed the course of the iodine in iodine-containing amino acids by autoradiography of paper chromatograms. Analogous procedures were used by Stepka, Benson & Calvin (66) after supplying $C^{14}O_2$ to algae. Similar methods have also been applied by F. K. Millar[5] to detect compounds which contain S^{35} in alfalfa leaf extracts. Phillips (69) has combined amino acids with 2,4-dinitrophenyl fluoride and chromatographed the resulting compounds, the detection of which is simple because they are yellow.

Some specific tests for amino acids are applicable to paper chromatograms. The sulfur amino acids may be treated (without affecting the other acids) with hydrogen peroxide (with or without molybdate as a catalyst) before chromatography (70). This treatment converts cystine and cysteine

to cysteic acid, methionine and methionine sulfoxide to methionine sulfone, these give more recognizable spots both as to position and intensity of color by the ninhydrin reaction. Winegard & Toennies (71) have sprayed paper chromatograms with a solution of potassium iodoplatinate which has a red color. Where the sulfur-containing amino acids occur the color is bleached. The oxidation products of the sulfur amino acids and other sulfur-containing compounds also react. Chargaff *et al.* (72) utilized the fact that sulfur-containing amino acids catalyze the oxidation of sodium azide by iodine. A known solution of iodine and sodium azide is sprayed on the paper and where the sulfur-amino acids are present, the iodine is reduced by the sodium azide.

Dent (70) has utilized an acid solution of *p*-dimethyl amino benzaldehyde to locate citrulline, and this may be applied to other ureides. Steward *et al.* (73) have also utilized this reaction to identify the ureide of γ-amino butyric acid in the potato tuber: this procedure may have general use for those amino acids from which ureides may be obtained.

Histidine has been located (46, 70, 74) with diazotized sulfanilic acid [Pauli reagent, cf. MacPherson (202)]. A precaution to observe here is that phenol must be removed because it also forms a colored compound.

An interesting development in the use of two-dimensional paper chromatography is due to Auclair (75) who used D-amino acid oxidase to detect D-amino acids. Auclair tested for keto acids, liberated by the oxidase, by the use of 2,4-dinitro-phenyl hydrazine. This was done on the papers after organic solvents were removed. The enzyme was sprayed on and incubated in a moist atmosphere for 4 to 6 hrs. Two control papers were used, one sprayed with enzyme but not incubated and one treated with ninhydrin in the usual way. By these methods, evidence for D-amino acids in insect blood was obtained [Jones (203) utilized D-amino acid oxidase to identify D-amino acids in polymyxin].

Procedure for identifying new compounds by paper partition chromatography.—Paper partition chromatography provides a powerful new tool for detecting and possibly proving new amino compounds in plant extracts or hydrolyzates. It is important, however, to observe certain precautions before postulating the presence of a new natural compound. Some of our views on this subject may be illustrated with reference to the discovery of γ-amino butyric acid as a normal constituent of the potato tuber and of most plants.

The first indication of a new amino compound is the discovery of one which does not correspond with the position of a known substance. However, a too ready assumption from position alone that a new amino compound exists, or even that all the substances present are identical with known compounds, may be misleading for the following reasons:

(*a*) Some spots will shift position depending on the pH of the solvents (36, 70). This is particularly true of some of the more basic amino acids.

(*b*) The presence of other substances such as salts and sugar may modify the movement of the amino acids.

(*c*) Some amino acids may also form more than one spot. Lysine at high

pH's separates into two spots (76). Aspartic and glutamic acid may tend to form double spots (70).

(*d*) Equally, one may miss a new amino compound because two different spots may superimpose, even after two-dimensional chromatography. For example, γ-amino butyric acid and methionine sulfoxide are not resolvable on a phenol:collidine-lutidine chromatogram; similarly, in the absence of ammonia, ornithine and glutamic acid are almost completely superimposed. The simplest test for such a pair of compounds is to try other chromatographing solvents. If a spot is not resolvable in any of a number of solvents this is, at least, presumptive evidence that it is homogeneous.

Granted evidence for a new amino compound that does not correspond in position to any known substance, what further steps are necessary or desirable to establish its identity? Some of the first procedures are as follows:

(*a*) Chromatograms are made under basic or acidic conditions to see if the compounds are basic, acidic, or neutral. Basic amino acids are more mobile under relatively basic conditions and acid amino acids are more mobile under acid conditions. Dibasic amino acids—glutamic and aspartic— move more slowly in phenol than the monobasic ones—norvaline and α-aminobutyric respectively. Diamino acids (e.g., lysine) move more slowly in the basic solvent than the corresponding monoamino acids (e.g., norleucine). Extracts may also be passed through cation or anion exchange resins to determine the acidity or basicity of the compound.

(*b*) Chromatograms are made after acid hydrolysis which decomposes peptides and amides.

(*c*) Chromatograms are repeated using copper carbonate in the phenol direction (77). This complexes the α-amino acids making them unreactive to ninhydrin and also makes the amino acids move differently on the chromatogram. It is difficult by this means to distinguish unambiguously between α- and β-amino acids, but the copper carbonate test works well for γ-amino acids as illustrated by the case of γ-amino butyric acid (73).

(*d*) Chromatograms are made after oxidation with hydrogen peroxide. After oxidation some sulfur compounds occupy different positions, but they may be detected in the normal way. Examples are the conversion of methionine and its sulfoxide to methionine sulfone which occupy the positions shown in FIG. II and the conversion of cysteine or cystine to cysteic acid.

(*e*) The ninhydrin gives some guide to possible identity. Proline and hydroxyproline, and presumably other imino acids, react red to yellow; β-alanine reacts light blue, turning later to purple. This behavior may, therefore, indicate an amino group in the β-position to a carboxyl. Since the characteristic brown color of the asparagine spot, in sharp contrast to that produced by glutamine, has not been explained on chemical grounds, it is difficult to conclude anything from a yellow-brown color produced by an unknown compound. The nature of certain yellow ninhydrin reaction products which often appear on the papers is still unknown.

(*f*) The position of a compound on a chromatogram may be compared with an extensive map (70).
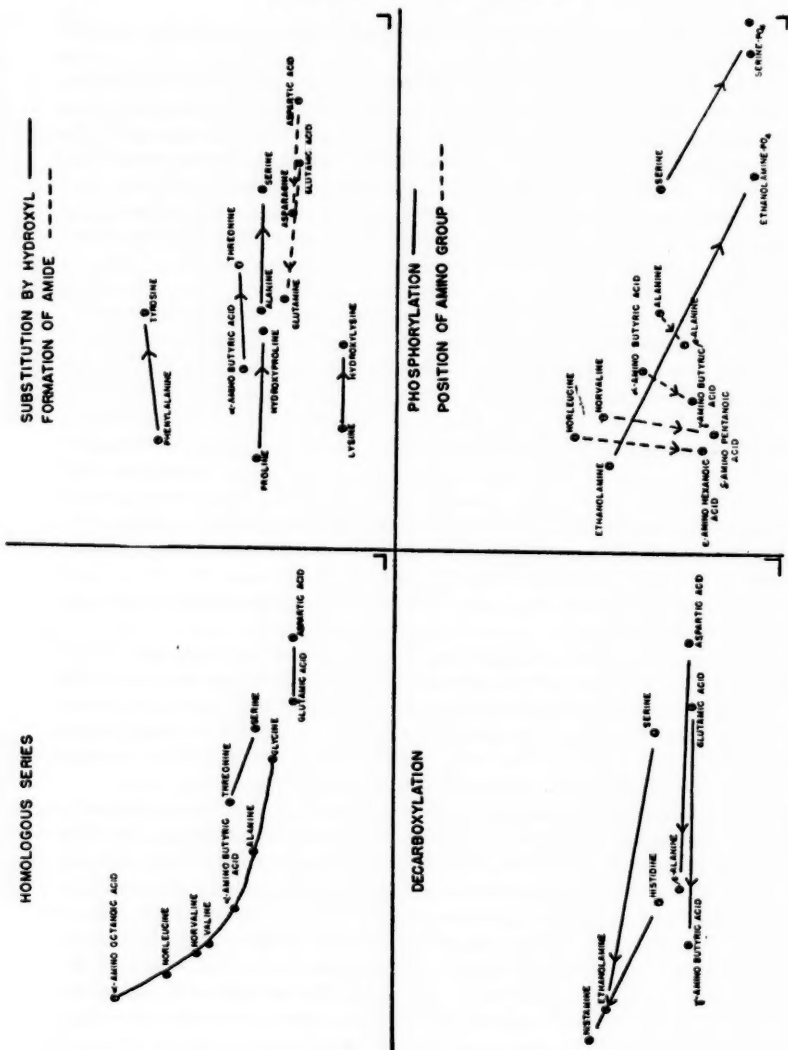
Fig. I. Effect of Molecular Configuration on Relative Positions of Chromatograms.

(g) The position of a compound on a chromatogram may be interpreted in terms of certain relationships which exist between the structure and movement of amino acids. In a homologous series (78), lengthening the carbon chain increases movement in both the organic solvents commonly used, so that homologues tend to lie on an arc on the papers. Examples of such series are: the α-amino acids (glycine, alanine, etc.); the dicarboxylic-amino acids (aspartic, glutamic, etc.); the diamino acids (ornithine, lysine, etc.); the β-hydroxy-α-amino acids (serine and threonine, etc.). In addition, certain changes in position attributable to the addition of a hydroxyl group or an amide or aromatic group, etc., may be helpful. For example, serine moves more slowly in phenol than alanine, tyrosine more slowly than phenylalanine, and "dopa" even more slowly. The amides, glutamine and asparagine, move more rapidly in phenol than glutamic and aspartic acids respectively. Substitution of hydrogen by phenyl confers mobility in both phenol and in collidine (cf. phenylalanine and alanine). Removal or addition of a carboxyl group affects the mobility in a predictable way (cf. glutamic with α- or γ-amino butyric acids; aspartic with α- or β-alanine). The position of the amino group relative to the carboxyl also affects mobility: mobility in phenol is increased, but in collidine decreased, by separation of the carboxyl and amino groups. These relationships, taken from Dent's map (70), are summarized in Fig. I.

(h) Selecting from possible natural compounds one may then compare the chromatography of those which might be identical with the unknown, bearing in mind the above rules; use may also be made of reactions which may be specific for a given grouping such as those given above. The chromatographic identity of an unknown and a known substance, in several solvents, is strong evidence that they are the same.

(i) However, final proof still demands the isolation of a homogenous product with which physical and chemical comparisons with known substances can be made. A procedure well adapted for these comparisons is x-ray diffraction (79). Electron diffraction patterns, though recommended by Polson et al. (80) are less easily obtained because the amino acids disintegrate in the electron beam. Infrared spectroscopy has possibilities which are as yet undeveloped. Chemical methods involve the formation of derivatives which, in the amounts easily obtainable from the paper, may need to be tested by chromatographic means. Steward et al. (73) reported that the ureide of γ-amino butyric acid from potato tuber was chromatographically identical with that prepared from the synthetic substance.

(j) Failure to discover any compound chromatographically similar to the unknown leaves one no recourse but the isolation and determination of structure by the classical methods of chemistry.

The soluble nitrogen fraction in the light of chromatography.—Partition chromatography may be used to confirm the presence of known amino acids in hydrolyzates and in the soluble nitrogen fraction of plants. It is, however, among the amino compounds which occur free that the outstanding advances have been made by chromatography.
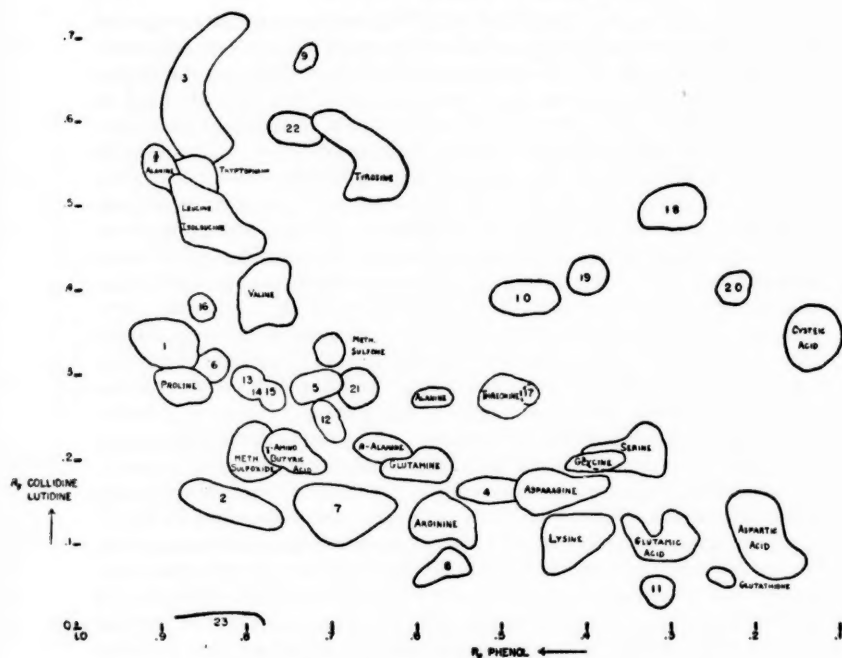
FIG. II. Map Showing the Location of Natural Ninhydrin
Reacting Nitrogen Compounds.

EXPLANATION OF FIG. II

Map showing location of natural Ninhydrin reacting Nitrogen compounds: based on calculated R_F values using alanine as an internal standard of reference. (Note: these positions apply when NH_4OH is omitted from the cabinets; phenol pH 3–5; redistilled collidine:lutidine 1:3.)

Materials used: Alcohol soluble nitrogen from asparagus shoot tips, bean fruits, carrot root, mushroom fruiting body, parsnip root, pea seeds, summer squash fruit, latex, and shoot of Ficus pumila;* hydrolysate of alcohol insoluble nitrogen from alfalfa leaves, and potato tubers.

Legend: Each zone indicates the area within which the midpoint of the spot may fall when the compound is in the different extracts. Well established nitrogen compounds, rather generally occurring free, are indicated by name. Cysteic acid does not occur free but is derived from cystine by oxidation: in some extracts it appears at a lower level opposite serine. Unknowns occurring free, or after acid hydrolysis of protein indicated by number. Histidine is not shown under these conditions.

1. Well defined violet spot, turning brown on standing; present in bean fruits in large amounts, but also present in tulip bulb, parsnip, mushroom, pea, and asparagus. Stable to acid hydrolysis.

* Material supplied by Prof. Knudson of Cornell University.

2. Violet spot, appearing in small amount in parsnip xylem, tulip bulb, pea, and asparagus. Stable to H_2O_2 but unstable to acid hydrolysis.
3. Pronounced yellow spot, appearing after ninhydrin; fairly widely distributed. It seems to be associated with decomposition of tryptophane.
4. Yellow to brown spot, persisting after acid hydrolysis; found in tulip.
5. Violet spot present in small amount clearly in mushroom and potato tuber and probably in squash seeds and asparagus; most probably α-amino-butyric acid.
6. Weak violet spot present in potato soluble-nitrogen.
7. Fairly strong violet spot present in squash seeds, pea, asparagus, tulip bulb, and mushroom. Revealed when phenol pH is about 2. Persists after oxidation.
8. Presumably basic substance giving a violet spot present in fair amount in mushroom and squash seeds; stable to hydrogen peroxide. Unstable to acid hydrolysis.
9. Well defined violet spot found in mushroom. Unstable on acid hydrolysis.
10. Rather diffuse faint violet area seen in parsnip; occupies same area as ethanol-amine.
11. Violet spot present in small amount in parsnip and probably in squash seed. It occupies the same area as hydroxylysine. α, ϵ diamino pinelic acid (207) also occurs hereabouts.
12. Well defined spot in tulip, removed by acid hydrolysis, stable to hydrogen peroxide.
13. Well defined light brown spot, turning violet upon standing, also present in tulip, but distinct from 12; removed by acid hydrolysis.
14. Well defined bright red-violet spot turning brown on standing; found in Ficus shoot homogenates.* Occupies a similar position to 13 and 15, but appears to be different from these.
15. Distinctive violet spot present in squash seed in relatively large amount, occupying the same location as 13.
16. Violet spot, present in small amount in squash seed. γ-hydroxy leucine appears in this general region.
17. Well defined pale yellow spot, occupying approximately the same position as threonine, found in small amount in tulip bulb.
18. Well defined faint blue spot from the acid hydrolysate of alcohol insoluble nitrogen of alfalfa leaves.
19. Weak violet spot also present in alfalfa hydrolysate occupying a position close to that of taurine but not apparently identifiable with it. The disappearance of 19 may be associated with appearance of a spot near to 9.
20. Weak violet spot also present in alfalfa hydrolysate.
21. Hydroxyproline: reddish-brown spot found only in protein hydrolysates.
22. Very conspicuous spot found in the latex of Ficus pumila.*
23. Violet spot—almost immobile in collidine—occurs in certain latex bearing plants.*
24. Weak violet spot only known in a single extract of summer squash fruit. This compound moves as far as leucine in phenol but definitely further in collidine:lutidine. A violet spot appears in the same general region in carrot and alfalfa hydrolysate.
25. Violet spot found in summer squash fruit occupying the position of β-alanine but not identical with it.
26. Violet spot found in hydrolysates of carrot and alfalfa moves slightly less in both phenol and collidine:lutidine than 9.

Dent, Stepka & Steward (81) were the first to apply successfully the two-dimensional paper chromatographic technique to the free amino compounds of plants. They found evidence for 21 known amino acids and amides and three unknown amino compounds in the potato tuber. All the amino acids commonly obtained by protein hydrolysis, except hydroxyproline, were found free. Relatively large amounts of asparagine and glutamine were found, as was to be expected after their isolation by Steward & Street (82). Aspartic and glutamic acids were prominent, as they seem to be in every extract of living plant cells or their protein hydrolysates.

One of the then unknown amino acids is a prominent constituent of the soluble nitrogen compounds [for analysis, see (73)]. This compound has been identified as γ-amino butyric acid (73); it is chromatographically identical with synthetic γ-aminobutyric acid; it is not affected by chromatography in the presence of copper carbonate; and it forms a ureide which is chromatographically identical with the ureide of the synthetic compound.

The number of published investigations on the amino compounds of plants by the methods of partition chromatography are few. Therefore, before reviewing these, the authors present a synopsis of the knowledge gained in this laboratory since the earlier paper[6] (81). The outstanding fact is that alcohol extracts of plant materials yield a variety of amino compounds. These include the compounds which were recognized earlier in the potato tuber as well as substances, some of them present in considerable quantity, which have not yet been identified. In order to show the number of these substances and something about their frequency of occurrence, the data of FIG. II have been compiled.

The map of naturally-occurring ninhydrin reacting compounds has been prepared in the following way: From each alcohol extract chromatograms were made using large enough amounts to reveal the substances present in smaller quantities. To compare the positions of the various compounds, alanine has been used as a reference substance. In our experience, however, one cannot establish the position of even a known amino acid absolutely by its R_F value alone, for in different extracts and for reasons which have been stated, they do not always occupy exactly the same position. There is, however, a restricted area within which each amino acid may be expected to fall and to indicate this the following has been done. Using only chromatograms under comparable and standard conditions (Phenol saturated with water, pH 3–6: collidine-lutidine (1:3), no ammonia or cyanide in the cabinets: temperature circa 25°C.) the center of each spot was determined and the R_F values so obtained were plotted and found to fall within the areas indicated on FIG. II. These areas bear no relation to the shape of the spots but show rather the range within which the center of the spot tends to fall. As the map shows, all the common amino acids and amides have their distinctive and easily recognizable positions. However, even a limited range of

[6] Work of R. M. Zacharius (Nutrition Foundation Fellow) and of F. K. Millar (A.E.C. Predoctoral Fellow).

familiar plant materials produces a surprising number of other ninhydrin reacting substances, several of which may occur in extracts of one plant. FIG. II indicates the existence of about 20 such substances which occur free and still await identification, and with at least three which were obtained on acid hydrolysis of the alcohol insoluble residue of alfalfa leaves. A potato protein hydrolysate produced no unexpected compounds.

Clearly, speculation upon the identity of so large a range of hitherto un-suspected plant constituents is premature. However, it may well be that many of these substances will find identification as decarboxylation products of already well known substances or they may be related to them in other ways, such as those indicated on p. 245. With so many substances occupy-ing closely similar positions, a too ready identification of the constituents of plant extracts by position alone is clearly liable to error.

On FIG. II the named amino acids[7] commonly occur free in plants. In Allsopp's (83) work many of these compounds were not recognized in all his samples. In greater or smaller amount, γ-amino-butyric acid is an invariable constituent of the soluble-nitrogen of all plants examined. It is therefore, the more surprising that Allsopp failed to mention it or to record it as an un-known. His selections include an interesting range of morphological ma-terials; however, conclusions on many of these should be reserved until it is established that the plants do not contain more nitrogenous constituents than Allsopp has yet reported.

Analyses of alcohol extracts of pollen and of the hydrolysates of the alco-hol insoluble residue show (84), in the main, the expected range of amino acids (in one instance glutamic was not reported), but with no obvious un-knowns. In this laboratory, hydroxyproline has never been found free in plants; therefore, if substantiated, its occurrence in pollen is interesting. Eny (85) presented a brief report on the amino acids in *Chlorella*. These re-sults are questionable because they present the anomaly that glutamine and asparagine are reported after hydrolysis. Hydrochloric acid digests of bac-teria and bacteriophage, which do not distinguish between free and combined amino acids, show a full range of the known amino acids (48, 86).

Other natural amino acids have recently been detected. Work (87) found α, ϵ diamino pimelic acid in the protein hydrolysate of *Cornybacterium diph-theriae*; α-amino-$\beta\beta$-dimethyl-γ-hydroxybutyric acid (pantonine) has been found (88) in acid-hydrolyzed *E. coli*, while α-amino-adipic acid has been found (53, 89) to be derived from lysine in liver and to occur in *Cholera vibrios* and to be a precursor of lysine in *Neurospora* (90). L-3,4 Dihydroxyphenyl-alanine is reported to occur in seeds (91).

Quantitative chromatography of amino compounds.—Most of the quantita-tive methods with paper chromatography have utilized the formation of a color with ninhydrine on the paper. Polson (80) dissolved the ninhydrin amino compound color in acetone and made colorimetric measurements.

[7] Fig. II refers to chromatograms made without ammonia in the cabinets; histidine does not appear as a spot under these circumstances.

Later (48) he used a method in which visible size and intensity of unknown spots was compared with that of standard spots run simultaneously. Awapara (92) and Naftalin (93) both used preliminary sprays with ninhydrin to locate the spots which were then cut out and the reaction was completed in the test tube. Since their chromatograms were only unidirectional, they are limited in the number of amino acids which can be separated. Bull *et al.* (54) and Block (94) have chromatographed one-dimensionally and they have measured the density [for instrument see (95)] of the ninhydrin color along the direction of movement. By integrating distance versus density, the total quantity of amino acids is estimated. Fisher *et al.* (96) make estimations only from size of spot, which seems to be inferior to the method of Bull *et al.* The two latter methods are also limited by the fact that they are one-dimensional.

Martin & Mittelman (61), after trying Folin's color reaction, microkjeldahl methods, and microtitration, resorted to polarographic determination of the copper complex of amino acids. Woiwod (97) adopted a similar procedure, except that he determined the copper complex colorimetrically. Both of these methods are one-dimensional and suffer further from the need of a test strip to locate the individual amino acids. Keston *et al.* (64) chromatographed one-dimensionally p-iodobenzene sulfonyl derivatives of amino acid in which the iodine was radioactive. The radioactivity was measured along the direction of movement of the solvent, and the intensity of radiation was integrated with distance to determine the amounts of amino acids. The quantitative possibilities in the two dimensional paper method have yet to be fully developed.

Synge (98) introduced the use of starch as solvent support in his work on peptides. Stein & Moore (50, 99) have carried the use of the starch column to a high degree of perfection for quantitative use. This method has been made capable of separating 15 amino acids from 2 to 3 mg. of material and appears to be quite accurate. Coupled with bioassay methods for amino acids present in only very small amounts, Stein & Moore's procedure should yield the best amino acid analyses of proteins now obtainable.

The column technique has been used by Blackburn (100) to separate the 2,4-dinitrophenyl derivatives of amino acids using silica gel support. Bell *et al.* (101) has used this method for proving the identity of α,γ-diaminobutyric acid obtained by hydrolysis of polymyxin.

Chromatography of soluble nitrogen compounds: some conclusions.—The widespread occurrence of amino compounds which cannot be identified with known substances indicates that our views on metabolic pathways may have been built upon a too limited range of possibilities. Some of these unidentified substances (e.g., Nos. 2, 9, 12, 13 in Fig. II) are decomposed by acid and may therefore be peptides or amides. Others, such as No. 4, Fig. II, are stable to hydrolysis. It is tempting, but premature, to speculate upon these substances or their rôle. Will previous suggestions (102, 103) that there are more plant amides than glutamine and asparagine be verified? May it be that the dicarboxylic acids, glutamic and aspartic, may somewhere in the plant kingdom form their diamides, which are known as synthetic sub-

stances? May isoglutamine and isoasparagine—also known as synthetic substances—occur naturally? These questions lend great interest to further investigation of the soluble nitrogen compounds. Clearly, however, our hitherto limited ideas on the range of compounds occurring in the soluble nitrogen fraction should be extended to include as possibilities decarboxylation products of the common amino acids as well as the amination products of commonly occuring metabolites, e.g., keto acids.

Further light on the sulfur-containing amino acids should follow from the combined chromatographic and autoradiographic techniques. Millar of this laboratory has examined alfalfa leaves which had been treated with $S^{35}O_2$ by M. D. Thomas. Different protein fractions (heat coagulum of the sap and a water, alcohol, and acid insoluble residue) yielded on hydrolysis a large range of amino acids of which three were found to correspond to methionine as sulfoxide and sulfone and cysteic acid, and to contain S^{35}. However, radioactivity due to S^{35} also occurred in nonamino compounds. This is mentioned to show the possibilities in tracing out the paths of sulphur amino acid metabolism [see McKee (11)] by the use of these methods.

METABOLIC RELATIONS OF AMINO ACIDS AND AMIDES

Reference may here be made to Steward & Street (1) and to the later and fuller treatments by McKee (11) and by Street (12).

Amino acid decarboxylation.—The identification by paper chromatography of γ-amino-butyric acid (γAB) as a constituent of potato tubers and many other plants is of interest because it could arise by decarboxylation of glutamic acid.

By the method of Schales (104) it has been found that the potato tuber has only minimal glutamic acid decarboxylase activity [18 c.mm. carbon dioxide per 30 min. per gm. fresh tissue—data quoted by Steward *et al.* (73)]. This small activity [cf. (104)] raises the possibility that γAB may not be a decarboxylation product of glutamic acid but rather its precursor by carboxylaton. Further evidence indicates that γAB is not found in protein hydrolysates and even the γAB which disappears when potato protein is synthesized does not reappear as such when the protein is hydrolyzed (73). This point has been tested by the authors with potato tissue submitted to conditions conducive to protein synthesis. In a given experiment with potato discs in aerated water, 60 per cent of the γAB disappeared during protein synthesis and did not reappear in this form on protein hydrolysis. Two possibilities exist: (*a*) the enzyme system responsible for peptide bond formation is absolutely specific for γ-amino acids, so that γAB would require to be carboxylated before it can be condensed, and (*b*) γAB can only furnish nitrogen for synthesis if it is first deaminated. The presence of free non-α-amino acids (e.g. β-alanine) in plants raises new problems in protein synthesis.

Since γAB is of general occurrence, it may be of great metabolic importance. The recognition of this compound will lend further interest to the investigation of carboxylation and decarboxylation which was earlier (1)

thought to play a rôle in determining the metabolism of potato tuber in the direction of protein synthesis, and may suggest new ways (through reversible amino acid decarboxylation) in which carbon dioxide may play a regulatory rôle.

Amino acids and amides.—Further work re-emphasizes the formation of amino acids and amides with the proteolysis characteristic of detached leaves (105, 106). According to Viets *et al.* (105), corn leaves exposed to calcium sulphate in the dark accumulate more glutamine than in the light. [cf. (1, p. 480) for cases of a contrary kind]; also Yemm (34a) finds glutamine, rather than asparagine, accumulation favored by high sugar content and light in barley leaves. Leaves supplied with nitrate or ammonia increase in soluble nitrogen compounds. Acid metabolizing plants like *Bryophyllum* decrease in organic acid and increase in amide in response to ammonia (107). Conversely, in nitrogen deficiency organic acids accumulate, malic acid in the case of peas (108), and when nitrate or ammonia are supplied to such deficient plants glutamic and aspartic acids and their amides accumulate. When ammonia is supplied, the increase is mainly of ammonia, asparagine, and glutamine: when nitrate is supplied in the light, protein is formed and only glutamic acid increased. Moyse (106) also associates glutamine increase with supply of ammonia to detached leaves. Following infiltration experiments, Kretovitch (109) emphasizes the accumulation of asparagine and ascribes this to the more ready utilization of glutamine. Ammonium sulphate labelled with N^{15} supplied to tomato plants enriches mainly their amino-dicarboxylic acids [glutamic acid more than aspartic acid (110)] and, in the roots of young plants, the amides [cf. (1), (111)].

It will be apparent that in the work on amino acids and amides cited above, there is evident confirmation of familiar results but little which is intrinsically new, either in fact or interpretation. Ammonia is again associated with a relatively greater increase in soluble nitrogen (particularly amides) and nitrate much more with increase of protein.

Amide synthesis.—Amide (glutamine) synthesis *in vitro*, from ammonia and glutamic acid, has been shown (112, 113, 114) under circumstances which suggest that it requires energy made available through adenosinetriphosphate (ATP). Elliott (113) speculated that phosphorylated glutamic acid was an intermediate. Presumably, similar events occur when glutamine is synthesized *in vivo*, from glutamic acid and ammonia. Examples of this include the striking one now used by Vickery (115) to prepare glutamine in bulk. Recalling the earlier work of Neish & Hibbert (116), it is almost certain that glutamine could not be obtained from beet tissue subjected to prolonged anaerobiosis—which would be intelligible in the light of recent results (112, 113, 114). There have been previous indications that a third amide might occur in plants [(102, (103) and cf. (12)]. Recently, however, the tendency has been to discredit this possibility. Street [(12, p. 417)] states ". . . the complete lack of positive evidence for other amides makes it almost certain that asparagine and glutamine are the only simple acid amides in higher plants." In view of the previously mentioned unidentified soluble

nitrogen compounds revealed by chromatography, some of them acid hydrolysable, this view can hardly be sustained.

Urea is apparently more widespread in plants than was hitherto believed. Following evidence for a third amide in certain seedlings, Damodaran *et al.* (102, 117), urea has been recognized as a major constituent, accounting for up to 50 per cent of the total amide in a particular case (117). Reifer & Melville (118) believe urea and urease to be widely distributed in plants.

Amino acids as external sources of nitrogen.—Numerous studies have been made of the use of amino acids as nitrogen sources for growing plants, for embryos or for tissue cultures (119 to 126). In many or all of these investigations inhibition of growth was observed with certain amino acids used singly—though glutamic and aspartic acids and alanine are notable exceptions (119, 122). According to Sanders & Burkholder (125), casein hydrolysates furnish physiologically balanced mixtures of amino acids which are more favorable to growth and differentiation than solutions of single acids.

Potassium-calcium effects in the utilization of amino acids.—The potassium-calcium effects on protein synthesis in the cells of potato tuber [(127), cf. (1)] have been reinvestigated (128) using the chromatographic technique to follow the soluble nitrogen compounds. Analyses of alcohol extracts were made after periods up to four days in aerated water, potassium-chloride, or calcium-chloride solutions.

The stimulating effect of potassium previously observed [cf. (1)] has been readily repeated (128), using the variety Katahdin. The depressing effect of calcium, however, was at first not obtained. Briefly, the effect of calcium now appears to be much more associated with the variety than was hitherto supposed from the earlier work with the King Edward variety. (The effect of calcium has been repeated using King Edward tubers from Canada, sufficiently to suggest that variety is more important than culture.) Indeed, a survey of a number of potato tuber varieties revealed interesting differences in their biochemistry as shown by their responses under these conditions. The persistence of aspartic and glutamic acids, under conditions in which all other amino acids decline as protein synthesis is stimulated by potassium, all points to a possible rôle of potassium in promoting the desamidation of glutamine and asparagine; but it would be unwise to conclude that this is the only point of contact between potassium and protein synthesis.

It is beyond the scope of this review to reconsider the nutritional interactions between potassium and nitrogen compounds with growing plants [cf. (1), and (12, p. 437)]. Some recent evidence of such interactions have appeared (129 to 134), but the ultimate explanation of the rôle of potassium in nitrogen metabolism is still obscure. The new methods of chromatography should permit a more intimate diagnosis than hitherto of the effects of mineral salts on the soluble nitrogen compounds.

Amino acids and photosynthesis.—Whether the metabolic pathways toward protein are the same in the green leaf in the light as in other protein synthesizing organs is still a major problem. Some light on this may be obtained from experiments done with $C^{14}O_2$ in Calvin's laboratory (135). The

technique, primarily designed for determining intermediates of photosynthesis, exposes *Scenedesmus* or *Chlorella* for short periods (30 sec.) to $C^{14}O_2$ and then, by use of partition chromatography, the C^{14} compounds are separated. The amino acid richest in C^{14} was aspartic acid, followed by alanine, asparagine, serine, β-alanine, and phenylalanine. Dark fixation of $C^{14}O_2$ shows that most of the amino acid radioactivity occurs again in aspartic acid and in alanine. The scheme of photosynthesis favored by Calvin & Benson (136), supposes an acetic-pyruvic-oxaloacetic-malic-fumaric-succinic-acetic acid cycle and all the radioactive amino acids formed in these experiments are derived directly or indirectly from the two ketoacids of the cycle. Glutamic acid, though conspicuous, was not radioactive, and this is supposed to indicate that the tricarboxylic acid cycle was not here involved.

OTHER COMPONENTS OF THE SOLUBLE NITROGEN FRACTION

Other than amino acids and amides the soluble nitrogen fraction of plants contains a variety of substances ranging from nitrate, in which the nitrogen is fully oxidized, to ammonia in which it is reduced. Among these, hydroxylamine and oximes may be intermediates in nitrate reduction or nitrogen fixation. Losses of gaseous nitrogen [for summary *see* (1)] have been attributed to interaction of nitrites in the cells with amino compounds. The only recent evidence on this subject (137, 138) tends to minimize the importance of this process, except under circumstances in which acidity developed in cells undergoing morbid changes.

Products of nitrate reduction and nitrogen fixation: the hydroxylamine-oxime hypothesis.—Steward & Street (1, p. 489) made brief reference to this hypothesis. Though it properly flows from work on microorganisms, the hypothesis has been extended until hydroxylamine and the oximes which it forms with keto acids could well occupy that central position in nitrogen metabolism of higher plants previously assigned to ammonia. In the light of recent work (112, 113, 114) showing an enzymatic reaction of hydroxylamine with glutamate to form the hydroxamic acid, the possibility should not be overlooked that hydroxylamine may also react with carboxyl groups. The hydroxamic acids on reduction would give amides. Recent reviews on nitrogen fixation (7, 139, 140) may be consulted.

New experimental advances in this field follow several main lines as follows:— (*a*) The formation of amino and oximino acids has been investigated in relation to the form (i.e. NO_3 or NH_3) in which nitrogen is supplied to microorganisms; (*b*) the effect of oxygen and anaerobiosis on oxime production during nitrogen fixation; (*c*) the direct supply of oxime nitrogen to plants. In Virtanen's laboratory (141 to 143) low nitrogen yeast (*Torulis utilis*) has been suspended in either ammonium sulphate solutions or in potassium nitrate solutions. Roine (143) found that, as expected, aminodicarboxylic acids and their amides were primary products in response to ammonia supply. Low nitrogen *T. utilis* stores less soluble nitrogen, but almost as much protein, from aerated nitrate solutions as from ammonium solutions (141). Oxime-nitrogen was formed by both normal and nitrogen

deficient yeast supplied with nitrate: the concentration of oxime-nitrogen in acid extracts of the cells reaching a maximum after 10 min. and declining thereafter. No oxime-nitrogen was formed in presence of ammonia, which is consistent with the view that oximes are normal intermediates of nitrate reduction. Conversely, Lascelles & Still (144) believe that in *E. coli* different enzymes are responsible for the reduction of nitrite and hydroxylamine on the one hand, and of nitrate on the other.

A number of recent observations bear upon the rôle of hydroxylamine. Pethica, Roberts & Winter (145) examined the effect of added hydroxylamine on fixation of nitrogen from N_2^{15} by *Azotobacter*. They attempted to distinguish between the direct inhibitory effect of this added substance, due to its rôle as an intermediate of fixation, and the indirect effects, due to its rôle as respiratory inhibitor. Since the two effects in question have not been demonstrated with the same cultures, no valid conclusion can be drawn. Novak & Wilson (146) even question whether the disappearance of added hydroxylamine from cultures of *Azotobacter* is any direct consequence of the metabolism of the bacterium since it also disappears from sterile medium. Segal & Wilson (147) also account for the use by *Azotobacter* of hydroxylamine as a nitrogen source in terms of the ammonia which arises from its decomposition.

From Virtanen's laboratory (148) has come a reconsideration of the hydroxylamine-oxime hypothesis in the light of anaerobic and aerobic nitrogen fixation by *Clostridium*. In contrast to aerobic nitrogen fixation, the anaerobic process yields no oxime-nitrogen. This led Virtanen to entertain the view that oximes may only arise from oxidation of ammonia but, by direct tests, oximes apparently arise more quickly from molecular nitrogen and nitrate than they do from ammonia. Thus, "the results do not speak in favor of the concept that oxime-nitrogen would arise only through oxidation of ammonia" and "oxidation of nitrogen, as a first step in nitrogen fixation leading on to hydroxylamine, is still considered to be plausible." The anaerobic fixation is conceived to pass through hydrazine to ammonia by reduction, leaving hydroxylamine to arise as a later oxidation product under aerobic conditions.

A direct approach to the hydroxylamine-oxime hypothesis has been made by Wood *et al.* Having synthesized 22 oximino derivatives, their toxicity and utilization by *Azotobacter* were tested (149). The α-oximino-dicarboxylic acids were nontoxic at 5×10^{-3} *M.*, whereas others were toxic in more dilute solutions. Both oximino-succinic and α-oximino-glutaric acids served as organic sources of nitrogen to *Azotobacter* in the absence of gaseous nitrogen. When supplied directly to oat plants by Wood & Hone (150) hydroxylamine and oximinopropionic acid were utilized to a certain extent in protein-synthesis, but the oximes of dicarboxylic acids (oximino succinic and glutaric) were much more effective sources of nitrogen for protein-synthesis, though still inferior to nitrate, and they are less toxic. These authors took pains to minimize the purely chemical decomposition of oximes in the external solution; they also changed the solution every three days to minimize the bac-

terial activity. However, since the plants were not aseptic, the oximes may have been absorbed only after they were transformed by microorganisms. It is thus possible, but not proven, that oximes are intermediates of normal nitrate reduction.

The principal excretion products from legume nodules, aspartic acid, oximinosuccinic acid with some β-alanine (151 to 153) are all consistent with the sequence oxaloacetic $\rightarrow$ oximino-succinic $\rightarrow$ aspartic acids. The later discovery of glutamic acid among the legume root-nodule excretion products (154) is suggestive of the probable rôle of α-keto glutaric acid as a direct acceptor of nitrogen, either in the form of ammonia or through the oxime. (Alternatively, glutamic acid could arise by transamination). Virtanen uses the formation of glutamic acid to support the idea that ammonia arises by oxidation from hydroxylamine, since it is oxaloacetic acid rather than α-ketoglutaric, which most readily reacts to form oxime. Burris & Wilson (155) consider that the similarity in the effects of $N_2{}^{15}$ and ammonia containing this isotope on *Azotobacter*, in which the isotope appears first (i.e., after a few minutes exposure) in glutamic and aspartic acids, lends support rather to the ammonia than to the hydroxylamine hypothesis. However, these hypotheses are not and cannot be mutually exclusive and the student of nitrogen metabolism of higher plants should still take into account that amino compounds may frequently originate by reaction of carbonyl and carboxyl groups with hydroxylamine followed by reduction of the oximes or hydroxyamic acids so formed.

An outstanding need is a critical reexamination of the soluble nitrogen fractions of root-nodules by methods of paper partition chromatography. A first step towards this end has been made by Hunt (156), who found in the alcohol extract 24 known substances and about 12 ninhydrin reacting but unidentified substances. From this it would appear probable that an undue emphasis may have been placed on the identifiable substances excreted to the solution. Zelitch, Burris & Wilson (157) have examined the hydrolysate of soybean root nodules which had been exposed to $N_2{}^{15}$ for short periods. Amino acid fractions were separated on starch columns and their N^{15} content determined. Valine was the richest in N^{15} and from this it was concluded that this acid might arise, as in the case of *Neurospora* (158), from its corresponding keto acid (α-keto-iso-valeric acid). However, what is needed is a determination, despite the difficulties, of the N^{15} content of the soluble-nitrogen fractions; one can hardly deduce from the distribution of nitrogen in the hydrolysate the form in which it entered the protein in synthesis.

Evidence has accrued on the rôle of heavy metals in nitrate reduction and nitrogen fixation [cf. (11, p. 14)]. The importance of molybdenum in nitrate reduction (as contrasted with ammonium assimilation) by higher plants has again been observed (159) and its rôle in nonsymbiotic nitrogen fixation emphasized (160 to 164). Anderson & Spencer (165), however, believe that very minute quantities of molybdenum permit nitrate utilization, while greater quantities are needed for symbiotic nitrogen fixation.

Nitrogen bases, alkaloids, etc.—The extensive sections dealing with alka-

loids in the reviews by Street (12) and McKee (11) may be consulted, as well as articles by Dawson (166, 167) which have appeared recently [cf. (1)]. James (168) continues to relate alkaloid synthesis to the products of protein breakdown and emphasizes that arginine and ornithine (identified in the free state in young shoots of *Atropa belladonna*) may be alkaloid precursors.

From the prevalence of decarboxylases for amino acids which contain a second polar group (169, 170) it may well be that free amines are more frequent in plants than has been supposed. Reference has already been made to the occurrence of γAB and β-alanine as decarboxylation products in higher plants. Ethanolamine,[8] known to occur in etiolated wheat seedlings (171), could arise from serine. A further case is that of histamine, produced by L-histidine decarboxylase in members of the *Chenopodiaceae* (172) and hydroxytyramine from dopa is also found. Emmelin & Feldberg (173, 184) have utilized the physiological activity of acetyl choline and histamine to detect these substances in the free state in the stinging nettle (*Urtica dioica*).

Growth-promoting substances.—It is to be expected that morphogenetic responses would be closely concerned with nitrogen metabolism. Although there are evident suggestions that the level of nitrogenous nutrition modifies such responses as those due to photoperiodism (175 to 178), there is still no sign that these responses may be causally related to any particular metabolite or metabolites. The effects of nitrogen compounds as growth-promoting substances will be dealt with in other reviews.

PEPTIDES, PROTEINS AND THEIR SYNTHESIS

Peptides.—In view of the number of as yet unidentified ninhydrin reactive substances, some of them acid labile, in common plants it may well be that peptides are of more frequent occurrence than has been supposed. Where peptides possess marked pathological or antibiotic activity, as in certain bacteria (179), there has been special reason for their investigation when they might otherwise have been overlooked. The present scanty knowledge of peptides in higher plants is, therefore, no indication either of their occurrence or importance.

A manuscript by Dekker, Stone & Fruton (180) redirects attention to older work (181, 182, 183) indicating that peptides are major constituents of certain marine algae. Fruton *et al.* have shown that the substance isolated from *Pelvetia*, when deamidated by alkali, yields a peptide consisting of three glutamic acid residues and they establish the presumption that in the plant the substance exists in the form of tri-L-glutamine. Other recent cases of naturally-occurring glutamic acid peptides are described (184, 185).

The protein-nitrogen fraction.—This subject will only be dealt with in outline, since it was the chief concern of McCalla (14) recently in the *Annual*

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[8] Ethanolamine is the logical precursor of choline, though the ability of higher plants to methylate it to choline has yet to be demonstrated [cf. (1, p. 487)]. It is therefore of interest that Steensholt (171) showed that etiolated wheat germ could use methionine as a methylating agent in the formation of creatine from added guanidine acetic acid, but could not methylate ethanolamine to choline.

Review of Biochemistry and has also received attention by both McKee (11) and Street (12). Lugg & Weller (186) have continued the studies of Lugg on the composition of leaf proteins. Proteins from four different plants (two grasses and two legumes) have almost identical proportions of arginine: lysine:histidine, which is consistent with the idea of a protein nucleus—or "Anlage"—to which reference has frequently been made [cf. (1)]. In the work of Wildman & Bonner (187), the striking fact which emerges is that a very large part of the protein of the leaf is recoverable in the form of an electrophoretically homogeneous protein. Therefore, the proteins of leaves may be more homogeneous and even similar from plant to plant, than might otherwise be supposed. According to these authors, it is the fraction which makes up the bulk of the leaf protein which contains the phosphatase and bound auxin and the smaller heterogeneous fraction contains all the other enzymes of the leaf. In the homogeneous protein fraction, 25 per cent of the phosphorus is labile "high energy phosphorus" (188). Wildman, Cheo & Bonner (189) have also investigated the increase of tobacco mosaic virus protein at the expense of the homogeneous protein fraction of tobacco leaf, which the virus apparently uses as substrate for its own synthesis. It will be recalled that Lugg & Best (190) investigated the effect of tobacco mosaic virus on the physical properties of the leaf protein.

Energy coupling in protein and peptide synthesis.—The problem of peptide bond synthesis and the coupling of energy to protein synthesis remains as crucial as at the time of the last review (1, p. 495). Evidence on glutamine and glutamic acid, as immediate donors of nitrogen in protein synthesis, has been obtained by Roine for yeast (191) and it is well known that glutamine is a growth factor for certain bacteria (11, p. 30; 12, p. 420). Whilst no attempt will be made to rediscuss the alternative ideas in the paths of protein synthesis, the following significant trends should be noted.

In the enzymatic synthesis of glutamine from glutamic acid and ammonia, the rôle of ATP as an energy donor is now well understood from work on liver homogenates (112, 113, 114). If glutamine does, in fact, function as an intermediate donor of nitrogen to protein, it may in part be embodied directly in the molecule [cf. isolation of amide from protein in well known work of Damodaran cited in (34a)]. Virtanen & Hamberg (192) show that the amide-nitrogen of zein could be hydrolyzed without hydrolyzing the peptide bonds, thus indicating the existence of combined amide in the protein and, after a measure of enzymatic hydrolysis, even more amide was obtained indicating that glutamine peptides are integral parts of the protein molecule. In liver homogenates, the rôle of ATP and magnesium in the enzymatic synthesis of peptides is now familiar from the work of Cohen & McGilvery (193, 194) as evidence of energy coupling in the formation of peptide bonds. A similar effect was demonstrated by Johnston & Bloch (195) who showed that incorporation of glycine marked with C^{14} into glutathione in pigeon liver extract only occurs in presence of a phosphate donor (ATP). In view of the previously mentioned (p. 253) effect of potassium and calcium on protein synthesis in potato discs (127), it is of particular interest that the activity of

the enzyme system producing peptide bonds which occurs in liver homogenates is stimulated by potassium and depressed by calcium (193). [A curious point is that calcium stimulated protein synthesis in the technique of Greenberg (199)]. Reexamining the rôle of phosphate bond energy in peptide bond formation, Lippman (196) presents a much more concrete picture than was hitherto possible:

Even without a complete knowledge of the finer mechanism of the condensations, it appears clear that a single energy-rich phosphate bond is used to effect a single peptide link. . . . Judging from the energy data the possibility would exist that one energy-rich phosphate bond could cover two or even more peptide links. This, however, would anyway be unlikely for reasons of reaction mechanism.

Clearly, it is of the utmost importance that these ideas be carried over to higher plants on the basis of demonstrable evidence. Albaum & Ogur (197) have isolated a substance from oats which may play a similar rôle to ATP but is not identical with it. More recently they have apparently isolated ATP itself (198).

Attempts to demonstrate protein synthesis *in vitro* by showing the incorporation of amino acids marked with C^{14} are now being made principally with animal preparations. Greenberg *et al.* (199) find that radioactive amino acids (glycine and alanine) are incorporated into protein in liver homogenates and tissue slices. This process is aerobic, inhibited by cyanide and azide, and though it does not respond to added ATP, there are indications [dinitrophenol inhibition (200)] that phosphate bond energy is involved. Borsook *et al.* (201) have extended this type of study to include the incorporation of a wide range of amino acids in proteins.

As these techniques become perfected and are applied to plant preparations, they will furnish the direct approach to the problem of protein synthesis which is needed.

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WATER RELATIONS OF PLANT CELLS AND TISSUES[1]

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Introduction

This review deals principally with the nature of the forces causing the movement of water into and out of plant cells and tissues. These forces are important because they control the distribution of water among the various tissues, thereby influencing turgidity and growth. They also are concerned in such special phenomena as exudation from wounds, root pressure, and guttation. Discussion of these forces naturally requires consideration of permeability and other phenomena related to cellular metabolism. Such processes as transpiration and the ascent of sap will not be discussed because they belong in the domain of the water relations of the entire plant. The absorption of water by intact transpiring plants is controlled largely by the rate of transpiration, hence it will receive only incidental attention. The reader is referred to Crafts, Currier & Stocking (1) for more detailed discussion of various phases of plant water relations and to Kramer (2) for a discussion of water absorption by plants.

The Classical Concept of Cell Water Relations

For more than a century the simple osmotic concept first proposed by Dutrochet in 1837 (3) has been considered adequate by most physiologists to explain the water relations of plants. According to this concept the cell was regarded as an osmometer in which the protoplasm functions as a passive, differentially permeable membrane separating the vacuolar sap from the external solution. Water movement was considered to occur solely along osmotic gradients, quite unaided by any activity of protoplasmic membranes. Such a simple physical explanation of water movement was welcomed during the 19th century when the trend was away from vague, vitalistic theories and toward definite mechanistic explanations. The most important modification of this early concept was the discovery that water movement from cell to cell occurs along diffusion pressure gradients rather than along gradients of osmotic pressure or concentration.

Until the past decade few people have seriously questioned the adequacy of this classical osmotic theory. It is true that certain exceptions to it have been noted. Stiles & Jorgensen (4) observed that water intake by pieces of

potato tissue had a Q_{10} of about three, a value much too high for a simple diffusion process. Ursprung (5) claimed that "the endodermis acts like a suction pump and reduction valve," because he found it to have a larger diffusion pressure deficit on the inner surface than on the outer surface which he believed caused water to be forced into the stele of the root. If such a difference exists it can be maintained only by the expenditure of metabolic energy by the endodermal cells. In recent years several investigators have suggested that water movement may be at least in part an electroosmotic process, caused by differences in electrical potential across the cell membrane. Effective differences in potential likewise could only be maintained by the expenditure of energy. None of these proposals has replaced the classical concept; nevertheless it is difficult to interpret some of the more recently published results on a purely osmotic basis.

TERMINOLOGY

Most plant physiologists have taken for granted the paramount importance of osmotic forces in plant water relations. Considerable attention has been given to clarifying the rather confused terminology applied to these osmotic quantities and Meyer (6) has recently published a concise discussion of a generally acceptable terminology. This is based on the assumption that water movement is primarily a diffusion process in which direction of movement is determined by gradients of diffusion pressure. It is impossible to measure the actual diffusion pressure of water and of solutions, but it is relatively easy to measure differences in diffusion pressure between pure water and solutions, because addition of solutes decreases the diffusion pressure of water by an amount equal in atmospheres to the osmotic pressure of the solution. This decrease in diffusion pressure (DP) of a solution is termed its diffusion pressure deficit, hereafter known as DPD. Since application of mechanical or hydrostatic pressure increases the DP of water, the development of wall pressure in a turgid cell causes an increase in the DP of its vacuolar sap.

In the range between full turgor and incipient plasmolysis the relations among the important osmotic quantities are expressed by the following equation: $DPD = OP - TP$ ($TP = WP$). DPD represents the diffusion pressure deficit of the cell which can vary from a value equal to the osmotic pressure of the cell sap (OP) to a value of zero as the turgor pressure (TP) increases. WP represents the wall pressure, which is numerically equal to the turgor pressure, TP, but exerted in the opposite direction. For a more complete discussion of the meaning of these terms the reader is referred to Meyer (6), Meyer & Anderson (7), and Crafts *et al.* (1).

Some attempts have been made to deal with the movement of water by means of the mathematical treatment used in thermodynamics. Edlefsen (8) has discussed the movement of water from soil to plant in terms of free energy gradients and Edlefsen & Anderson (9) have applied this treatment to soil moisture analysis. Broyer (10) recently published a detailed thermodynamic analysis of cell water relations. This treatment is in accord with the

growing realization that water movement is always along gradients of decreasing free energy. The free energy of water may be changed in various ways, as by gravitational, electrical, and adsorptive forces, the imposition of hydrostatic pressure, and the addition of solutes. While the thermodynamic treatment is useful for certain analytical studies, the expression of free energy gradients in terms of DPD, measured in atmospheres, is just as satisfactory for most physiological work, and more easily understood by most plant scientists.

A theroretical interpretation of turgor pressure has been proposed by Burström (11), in which TP is regarded as a driving force tending to cause water intake by the cell. Only at equilibrium states is it defined as equal to wall pressure; under conditions of water uptake TP is considered to decrease as wall pressure increases. In the sense of driving force, this quantity in our terminology would be called the DPD difference or DPD gradient, but when Burström defines turgor pressure as equal to $O - E$, where O = osmotic pressure of the cell sap, and E = osmotic pressure of the external solution, then TP has the meaning of a potential driving force due only to the effect of solute. Since he also defines TP as equal to $S - E + W$ (where S = suction of the cell, W = wall pressure) it assumes the meaning of an actual pressure, because in S the hydrostatic pressure is operative on the diffusion pressure of water. It does not seem advisable to discard the well-established meaning of TP as an actual hydrostatic pressure, equal to the counter wall pressure at all states.

WATER BALANCE IN PLANT CELLS

The relationship between the three osmotic quantities, $DPD = OP - TP$, indicates that the turgor pressure (TP) opposes those forces (OP) which account for the movement of water into the cell. In other words OP tends to increase, TP to decrease, the water absorbing power of the cell. The term OP here is used broadly to include the effects of solutes, imbibants, and, where they might exist, any "active" forces. It is clear that the presence of solute in the external solution opposes inward movement of water. This factor has recently been evaluated by Broyer (10) and by Burström (11). If the external solute concentration is sufficiently high, water leaving the vacuole causes separation of the protoplast from the cell wall, i.e., plasmolysis. Negative pressure (tension), such as that which develops in the xylem conductors of transpiring plants, also reduces the DP of water and opposes inward movement. Thus, there is competition for water between intracellular and external forces, and Broyer (10) has expressed this as $NIF = \Sigma IF - \Sigma EF$ or, net influx specific free energy = sum of influx specific free energies − sum of efflux specific free energies.

Osmotic versus imbibitional forces.—Plant physiologists find the term imbibition pressure a useful one [Seifriz (12), presents the case well] to identify the tendency of colloidal imbibants to reduce the DP of water. This is in contrast to osmotic pressure, the effect of solutes in reducing DP. While the two quantities at their extremes (salt solution *vs.* air dry gelatin, for

example) are easily distinguishable, in a heterogeneous system such as vacuolar sap the distinction becomes more arbitrary, and, to some, unnecessary [Brooks & Brooks (13)]. Actually the ultimate forces involved must be quite similar. These forces include various types of bonding—ionic, covalent, dipole, and hydrogen bonds, all of which reduce the diffusion pressure of water. In a steady state condition, a dynamic equilibrium exists between these forces, tending to equalize the DP of water in all parts of the cell.

Hydration of cellular constituents.—Water retention by cell walls is believed to result mainly from hydrogen bonding by hydroxyl groups exposed on the surfaces of cellulose micelles, to microcapillary forces, and to complex swelling of hydrophilic, infiltrating materials.

The water relations of protoplasm are of significant importance, but the exact mechanisms whereby water is absorbed and retained are not too well known. Any adequate explanation must be able to account for the characteristic physical properties of the living substance, and for its ability to function under widely varying water contents, to some degree independent of solute content. The reader is referred to Sponsler & Bath (14); Seifriz (12); and Frey-Wyssling (15) for discussions of the problem. Water is held in the protoplasm by hydrophilic groups on polypeptide backbones and side chains by means of hydrogen bonding, as connecting bridges between chains, and mechanically between the polypeptide framework meshes (microcapillary water). Dehydration may involve gradual narrowing of the meshes, with the masking of hydrophilic groups by lipids. Salts and other solutes impart to and regulate osmotic forces of the protoplasm and by direct or indirect means they may cause increased or decreased hydration.

In vacuoles, water-retaining forces are largely osmotic in nature, although colloidal substances (soluble protein, mucilages, etc.,) are generally present, and hold adsorbed water on their surfaces. Evidence that there may be active (nonosmotic) forces increasing the water content of vacuoles will be considered in the next section.

Consideration of the structure and unusual properties of water makes its behavior in hydration phenomena of tissues more understandable. The current view is that instead of a mass of individual molecules randomly arranged, the molecules are attracted to each other in a sort of lattice-like network with bonds constantly shifting. A high degree of molecular polarity and a tendency to form hydrogen bonds account for the unique solvent properties of water as well as for its ability to hydrate colloids.

Factors affecting water balance.—Water balance in plant cells can be upset by many different kinds of reactions or processes such as changes in permeability, swelling of the cytoplasm (vacuolar contraction), metabolic reactions such as change of starch to sugar, transfer of water to adjacent cells, and transpiration. Chloroplasts of *Spirogyra* have been observed to lose water to the rest of the cell by contracting [Osterhout (16)]. Swelling of the cytoplasm and nuclei is characteristic of initial stages in killing by certain herbicides, followed by dehydration as irreversible injury occurs [Currier (17)].

The cell sap concentration is known to vary according to changes in the

environment, an increase in moisture stress usually causing an increase in osmotic pressure of the cell sap. Gasser (18) studied the sap concentration of plants which were kept under conditions of an extended water deficit. Out of 95 plant species 69 showed an increase of osmotically active solutes of from 15 to 223 per cent of the initial value. Similarly, a shift in the other direction could be demonstrated where the environment is nearly saturated with water vapor. Other factors controlling water balance in cells will be considered in the subsequent discussions.

Autonomic rhythms.—Certain well known periodicities in plant behavior are becoming more clearly understood as true endogenous rhythms, demonstrable under constant environmental conditions. Many of them reflect a changing water balance of cells.

Detopped root systems exhibit periodic fluctuations in root pressure and exudation [Grossenbacher (19); Skoog, Broyer & Grossenbacher (20); Speidel (21); Daniel (22)]. The mechanism underlying this rhythm is obscure; explanations advanced have included changes in permeability, in osmotic pressure, in enzyme activity, in respirable substrate, and in active water uptake. Detopped root systems exhibit similar diurnal fluctuations in absorption of water through the stumps, i.e., negative exudation [Hagan (23)]. The cycles were observed in roots under the influence of water deficit, both in soil and in air, and were dependent upon adequate oxygen supply. This demonstrates the existence of a mechanism in roots by which water content varies periodically, and it suggests a variable hydration of protoplasm. Inequalities between absorption and exudation of water by isolated onion roots [Rosene (24)] are probably a reflection of the same mechanism. This recalls the observation of Shreve (25) that regular diurnal changes in water-holding capacity of cactus tissue occur, the water-holding capacity being greater during the day than at night.

Under constant environmental conditions periodicities have been observed in transpiration [Biale (26); Montermoso & Davis (27)]; in guard cell movement [Sayre (28); Tagawa (29)]; and in physical properties of leaf cuticle [Fogg (30)] inferring a fluctuating cuticular transpiration. Härtel (31) believes that there is an active regulation of cuticular transpiration through metabolically induced changes in pH, which produce swelling changes in the epidermal wall.

Turgor movements motivated by an endogenous rhythm are considered by Bünning (32) as due to changes in pH of the cell, which are brought about by endogenous variations in respiratory rate and in carbon dioxide production. Guard cell movement and cell elongation are similarly interpreted, the pH change acting by changing the permeability to water of the cell membranes. The periodic rotating movements of the leaflets of *Desmodium gyrans*, the object of much research by Bose (118), have been reinvestigated with respect to energy mechanisms by D. M. Bose, Dutt & Guha-Thakurta (33). Fluctuations in respiratory rate as indicated by oxygen utilization were observed to be synchronized with these turgor movements, which occurred at a rate of one to four minutes each. Krishna Iyengar (34)

reported a fluctuating water content of whole young plants, and suggested pH changes as the underlying cause.

The basis for all of these rhythms lies in metabolic processes in the cytoplasm. An inherent rhythm in myxomycete protoplasm [Kamiya (35)] was demonstrated with a unique apparatus whereby the direction of streaming was controlled by application of hydrostatic pressure. The periods were about two minutes long. It is the view of Seifriz (36) that the phenomenon is one of rhythmic contractility, involving folding and shortening of polypeptide chains, and that the property may be a fundamental and generally occurring property of protoplasm. Contraction may in some way be related to changes in hydration of the chains.

Stålfelt (37) demonstrated that the viscosity of protoplasm in *Helodea densa* cells is not constant but varies throughout the day and night. The fluctuations occur in periods of about 2 hr., but disappear after three days in constant darkness. It was concluded that the changes are initiated and maintained by diurnal fluctuations in light, but that they are not directly associated with photosynthesis. Interpreted with regard to the views of Kessler & Ruhland (38), light might influence the charge on protoplasmic particles, which in turn would affect hydration and viscosity.

The examples just considered make it clear that water balance, far from being a static or even a steady state condition, may be constantly changing. Endogenous fluctuation in hydration reflects an intimate relation between water absorption and metabolic processes.

EVIDENCES OF ACTIVE OR NONOSMOTIC ABSORPTION

During the past decade widespread doubts have arisen concerning the adequacy of a simple osmotic theory to explain certain phenomena encountered in studies of cell water relations. Considerable evidence has been presented which can be interpreted as indicating that cells sometimes absorb more water than would be possible by simple diffusion, because the uptake must have occurred against a diffusion pressure gradient. Such occurrences can be explained by assuming that the protoplasm actually secretes water into the vacuole analogous to the way it is believed to bring about the accumulation of solutes. Absorption of water under such conditions must require the expenditure of energy, hence it must be directly related to cell metabolism and particularly to respiration. It has therefore been proposed that a "nonosmotic" force is involved in the water relations of cells. Some of the evidence for the existence of an active or nonosmotic force will now be considered.

Discrepanices between cryoscopic and plasmolytic measurements.—The whole question as to the validity of the classical view was reopened in 1936 by Bennet-Clark, Greenwood & Barker (39) because they found lower cryoscopic than plasmolytic values of osmotic pressure for the same tissue. Emphasizing the permeable nature of the plasma membranes, they proposed that the tonoplast "secretes" water into the vacuole instead of functioning passively in restraining solute and permitting passage of water. They cal-

culated secretion pressures as high as five atmospheres. On the other hand, in some tissues there was good agreement between the two determinations. While these discrepancies have been confirmed [Mason & Phillis (40); Roberts & Styles (41); Bennet-Clark & Bexon (42); Currier (43)] for certain plants, there has been little positive evidence to support the secretion hypothesis. The failure of young beet root to show a significant discrepancy, in contrast to older dormant roots [Currier (43)] is not in line with the expectation that water secretion should be found in actively growing tissues.

For an explanation of the discrepancy one may look to the potential errors inherent in both methods for determining osmotic pressures. Despite its numerous difficulties the plasmolytic method remains a valuable one. While the measurement is made at incipient plasmolysis, volume changes are easily obtained and OP values in other states of water balance can be calculated. Volume changes from normal to incipient plasmolysis are not great (about 5 per cent for storage parenchyma) and little error is involved in the measurement. Volume changes of a greater order have been reported, but here it is possible that strong plasmolyzing solutions caused the tissue to shrink below its volume at incipient plasmolysis. Physiological changes in the cells due to sectioning and plasmolyzing are of course of constant concern. For example, increased respiratory rates upon plasmolysis are known to occur [Bennet-Clark & Bexon (44)], which may be an indication of an over-all modified metabolism. Errors in the plasmolytic method caused by adhesion of the cytoplasm to the wall [Buhmann (45)], penetration of plasmolyzing solute into the cells [Eaton (46)], and measuring volume changes too low [Levitt (47)] probably are unimportant compared to errors inherent in the cryoscopic method.

In cryoscopy, although freezing points are determined with great accuracy, the use of sap expressed from tissue constitutes a possible error of serious proportions. Too great confidence has been placed in the belief that killing of the tissue and destruction of the plasma membranes by cold or heat permits the expression of unaltered vacuolar sap. Tests on frozen and thawed red beet root [Currier (48)] show that as pressures are increased, increments of sap exhibit declining freezing points. It seems reasonable that sap expression would result both in filtration of solutes and in release of dilute solution from protoplasm and cell walls. The lower cryoscopic values may thus be explained.

Dilution of expressed sap by colloidally imbibed liquid [Roberts & Styles (41); Currier (43)] as an explanation of plasmolytic-cryoscopic discrepancies is held improbable by Levitt (47) since he believes the volume of this contaminating liquid insufficient to explain differences of many atmospheres. Since protoplasmic volume can vary, and protein analysis gives only an approximation, it is not unlikely that sap expressed from the protoplasm is a cause of the discrepancy. Additional work is needed to assess the relative importance of imbibitional water and filtration of crystalloid solutes in contributing to low cryoscopic values, especially where discrepancies are high. We are in accord with Levitt that the dilute solutions pressed from

living tissues by Mason & Phillis (40) and Bennet-Clark & Bexon (42), and regarded by them as vacuolar sap, suffered from filtration.

Kerr & Anderson (49) observed that while the osmotic pressure of sap expressed from young cotton seeds exceeded their DPD as measured gravimetrically, in seeds 24 days old the DPD exceeded the osmotic pressure, the difference increasing with age of the seeds. Neither the DPD nor the amount of water absorbed was decreased by potassium cyanide or other treatments which inhibit respiration, and it was concluded that the vacuolar sap probably was contaminated with sap from the cytoplasm, released by freezing prior to expression of sap. In this connection there are some interesting remarks by Ubbelohde (50) to the effect that, due to various degrees of association, water inside an intact cell may differ significantly from the water in the sap squeezed out of it.

The energy requirements of water secretion have been considered by Levitt (47), who concluded that it is quite improbable that secretion pressures of the magnitude sometimes claimed can occur in plant cells, and that the evidence for water secretion is invalid because it was incorrectly interpreted. While Levitt's conclusions are probably correct it appears that too much confidence cannot be placed in the values used for specific area (tonoplast area) in the calculations. On the molecular level, it could hardly be considered that every point of the surface would be active in secreting water and a factor of 10 here would modify the problem considerably. The effective area might well change periodically, which might be reflected in changes in permeability. Bennet-Clark (51) suggests that the permeability constant used by Levitt $(20 \times 10^{-4}$ cm. per hr. per atm.) in his calculations may be too high because few measurements have been made, and these under questionable conditions. The representative data given for plant cells by Brooks & Brooks (13) indicate, at least, a wide variation in water permeability.

To sum up, on the basis of comparing plasmolytic and cryoscopic values there is no clear cut-evidence for water secretion.

Respiration and water intake.—Since any nonosmotic absorption of water by plant cells requires the expenditure of energy, it presumably must be closely related to the rate of respiration. There is agreement that water intake is somehow related to the presence of oxygen. The evidence comes principally from studies on three kinds of tissue: potato tuber and other storage parenchyma, roots and root systems, and *Avena* coleoptile segments. Reinders (52, 53) reported that water absorption by potato tissue is dependent on an adequate supply of oxygen. Treatment with auxin caused an increase in both respiration and water intake. She concluded that water absorption may not be directly connected to respiration, but that it was likely a secondary effect of an increased solute concentration. Steward, Stout & Preston (54) criticized Reinders for measuring respiration by change in dry weight, and commented that the fact that a treatment increases both respiration and water intake does not prove that increased respiration is the cause of increased water intake. They note, however, that in aerated solutions potassium salts stimulate and calcium salts depress water absorption

in a manner not wholly explicable as an osmotic phenomenon. They finally suggest

that actively metabolizing cells which can grow, may absorb water in a manner which has but little relation to any conventional osmotic or suction pressure theory, but may be more directly linked with metabolic processes (respiration and protein synthesis); processes which are determined by oxygen and affected by the nature of the salts present in the external solution.

Rosene (55) found that water absorption and exudation of individual onion roots were reversibly inhibited by potassium cyanide. In a later study (56) she found that about three-fourths of respiration and of exudation by onion roots in water were inhibited by azide. These results were interpreted as indicating the direct utilization of metabolic energy in the absorption of water and development of exudation pressure in onion roots. Van Overbeek (57) studied entire tomato root systems grown in nutrient solution. The osmotic pressure of exudate from detopped root systems was considerably lower than the osmotic pressure of the mannitol solution in which the roots were immersed to stop exudation. This difference was considered to be a measure of the active or nonosmotic component of the water absorption mechanism which amounted to 50 to 70 per cent of the total DPD. It was reversibly inhibited by 10^{-4} M KCN, indicating its probable dependence on respiration as a source of energy. Kelly (58) found that water is absorbed by oat coleoptiles only under aerobic conditions, and absorption is inhibited by substances which inhibit respiration.

Some believe that it is the active component of water intake that is increased by oxygen. Brauner & Hasman (59) made this suggestion with regard to carrot root tissue. The disappearance of fluctuations in exudation [Grossenbacher (19)] and in water uptake by stumps of detopped root systems [Hagan (23)] under anaerobic conditions is offered as evidence that the causative force is associated with respiratory energy. Skoog, Broyer & Grossenbacher (20) could find no definite correlation between changes in rate of exudation from *Helianthus* roots and rate of respiration, although they believed that exudation is related to respiration in some manner.

Although there clearly seems to be a relation between respiration and water absorption of tissues and isolated roots or detopped root systems this relation is not evident in intact transpiring plants. Water intake by isolated tissue or root systems is controlled by forces resident in the tissues or organs themselves, but water intake by roots of transpiring plants is a passive process controlled primarily by the rate of transpiration. Passive absorption so far exceeds active absorption, that changes in the latter are often not detectible. Wilson & Kramer (60), for example, found no relation between oxygen consumption of the roots and water intake by intact transpiring tomato plants.

Levitt's (47) view, referred to previously, is that active or nonosmotic gradients of the magnitude sometimes claimed are thermodynamically impossible. Calculations showed that respiration could not provide enough

energy to maintain a nonosmotic gradient of more than one atmosphere between the cytoplasm and the vacuole of a cell. Levitt regards maintenance of a nonosmotic gradient of one atmosphere across the cortex of a root as quite possible, however, because of the much lower ratio of surface to volume in an entire root than in a single cell.

Even if a causal relationship between respiration and water intake were proven we would still wish to know how an increase in respiration can cause an increase in water intake. There is as yet insufficient direct proof that the effect of oxygen and of respiration is on an active water movement. It is possible for example, that variations in metabolic activity might change the permeability of protoplasmic membranes or the concentration of solutes in the vacuole enough to affect osmotic movement of water.

Auxin and water intake.—Several investigators have reported that auxin increases the absorption of water by plant tissue. Skoog, Broyer & Grossenbacher (20) found that indoleacetic acid increased the rate of exudation from pea and sunflower root systems. No correlation could be established, however, between auxin activity or diurnal cycles in exudation and total respiratory rates. According to Reinders (53), addition of auxin results in increased water intake by slices of potato tissue under aerobic conditions, but not under anaerobic conditions. She suggested that auxin increases water intake by hydrolyzing starch to sugar, thereby increasing the concentration of osmotically active materials in the cells. Commoner, Fogel & Muller (61) attempted to determine whether auxin increases water absorption of potato tissue by increasing plastic extensibility of the cell walls or by stimulating the active solute absorption mechanism. They concluded that auxin causes increased accumulation of salt in the vacuoles, resulting in increased water absorption and high turgor pressure. This view seems to be erroneous, because van Overbeek (62) demonstrated that auxin caused increased water absorption by potato tissue in distilled water where intake of solutes was impossible. Furthermore, the sap expressed from auxin-treated tissue had a lower osmotic pressure than that from control tissue receiving no auxin. Evidently auxin-induced water absorption cannot be caused either by salt absorption or by starch hydrolysis. Possible causes are an auxin-induced decrease in wall pressure resulting from increased plastic extensibility or active growth of the walls, or an auxin-induced increase in active or nonosmotic water absorption.

If a decreased wall pressure is responsible, the findings of Commoner *et al.* (61) that auxin (in the presence of potassium chloride or potassium fumarate) can induce water uptake by potato tissue from hypertonic solutions is unexplained. The need of further investigating the uptake of water from slightly but definitely hypertonic solutions is indicated.

Kelly (58) found that auxin increased both respiration and water absorption of oat coleoptiles under aerobic conditions, but not under anaerobic conditions. Furthermore it was not effective in the presence of respiratory inhibitors such as azide and iodoacetate. This disagrees with results of Levitt

(63) who found that potassium cyanide did not reduce auxin-induced uptake of water by potato tissue. Perhaps it is not to be expected that potato tuber tissue and actively growing coleoptile segments would behave similarly under these conditions. Levitt also found that reducing the temperature from about 25°C. to almost freezing did not result in loss of water, and there was no difference between the samples in auxin solution and in distilled water. But Reinders (53) found less water absorbed at low temperatures, where no auxin was added. There is no agreement, therefore, concerning dependence of auxin-induced water intake on energy supplied by respiration. For these reasons, and because no equilibrium point was attained—the potato tissue continues to absorb water indefinitely—Levitt concluded that auxin-induced water intake is not caused by active water absorption. The other possibilities are increased osmotic pressure, disproved by Van Overbeek, increased protoplasmic hydration, and decreased wall pressure. Levitt calculated that an increase in water content of only five per cent would more than double the hydration of the protoplasm above that occurring in distilled water. Such an increase in hydration seems very improbable. He concluded that auxin-induced water intake can be explained only by the fact that auxin causes increased plasticity of the cell walls, permitting indefinite increase in volume. If the walls are rigid, the wall pressure rapidly increases as water enters, soon reducing the DPD to zero, but if the walls are relatively plastic the continued increase in volume will prevent the wall pressure from becoming high enough to stop absorption.

Zinc deficient plants have been found to be low in auxin (Tsui, 64), suggesting that this might be the reason for the low water content and slow growth characteristic of such plants. Lack of auxin, Tsui suggested, might decrease extensibility of the cell walls, resulting in a lower DPD and less water absorption than in plants synthesizing normal amounts of auxin.

There is thus a conflicting body of evidence surrounding the problem of water uptake by tissues, as it relates to the presence and action of auxin, to respiration, and to growth. The mechanism governing the enlargement or elongation of cells is one of the great unsolved problems in plant physiology. A critical reaction in the process remains obscure.

That the primary effect of auxin is an increased plastic extensibility of the cell wall has been the view of Heyn (65) and others, working for the most part with the *Avena* coleoptile. There are also a number of theories involving other factors as primary or immediate causes. Among the factors suggested are increased cytoplasmic permeability, swelling of intermicellar pectic colloids, active wall growth, increased solute accumulation, water secretion, and swelling of protoplasm. The data are extensive and the problem requires a separate treatment. It is difficult to see how permeability changes by themselves, as suggested by Pohl (66) and others, can be considered to be the primary cause, since here only rates are affected, and permeability to water is already relatively great. In a recent review, Audus (67) concludes that we must either explain a number of separate effects produced by auxin in dif-

ferent tissues, or we must base our theory on some more fundamental action, or "master reaction" in the sense of Thimann (68). Northen's (69) report of dissociation of protoplasmic proteins by auxin is given as an example.

Electroosmosis.—Various workers have proposed that electroosmosis might cause the transport of water against diffusion pressure gradients. The early work has been summarized by Keller (70) and by Heyl (71). The latter believed that root pressure is caused by electroosmosis, the difference in potential being maintained by expenditure of metabolic energy. Lund (72) believed that the potential differences which he found in roots of woody plants might be large enough to cause inward and upward movement of water and the development of root pressure. Bennet-Clark & Bexon (44, 73) have proposed an electroosmotic mechanism to explain the movement of water across the cytoplasm into the vacuole. Brauner & Hasman (59, 74) and Studener (75) support the view that there is an electroosmotic component in the uptake of water by bulk storage tissue.

It is well known that liquids can move across certain types of membranes under the influence of an applied electric current. The volume of flow is proportional to the difference in potential and the direction of flow of water is usually toward the negatively charged side of the membrane. This has been experimentally demonstrated for membranes of wood by Stamm (76), and Stern (77) caused water to flow through segments of willow twigs. Electrical potentials have been demonstrated in many plant and animal tissues, and across the membranes of large plant cells. Blinks (78) discussed the factors affecting the magnitude of potential differences in *Halicystis*, *Valonia*, and *Nitella*. His work indicates that they are definitely related to the metabolic activity of the cells, though the relation is closer in *Halicystis* and *Valonia* than in *Nitella*. The difficulty arises in explaining how a continuous difference in potential can be maintained between the exterior and the interior of the cell. Most investigators seem to depend on differential permeability to various ions to explain such a mechanism, or they postulate continuous production of some such substance as bicarbonate ion which accumulates at the inner membrane surface. Bennet-Clark and Bexon (73) for example, concluded from experiments on onion epidermis that electroosmosis resulted from more rapid diffusion of K^+ than Cl^- through the negatively charged cell membranes. No energy was directly expended in this instance, but if the protoplasm expends energy in maintaining a potential difference, as it does in at least some cells, then electroosmosis must be regarded as a form of active water absorption.

Using the gravimetric method for DPD, and working with bulk tissues of potato tuber, and carrot and beet roots, Brauner & Hasman (59, 74) found the DPD to be lower in solutions of calcium chloride and magnesium chloride than in isotonic sucrose solutions. The gravimetric method involves immersing weighed tissue blocks in graduated solutions. The osmotic pressure of that solution which causes no change in weight of the tissue is considered to be equal to the DPD. The results of Brauner & Hasman mean that tissues in osmotic equilibrium with a sucrose solution lose weight (assumed to be

caused by loss of water) when transferred to an isotonic calcium chloride solution. They concluded that cations moving into cation-permeable membranes discharge the zeta potential within the membrane pores, thus reducing or eliminating the electroosmotic component. Considering the possibility of changes in protoplasmic hydration and the likelihood that calcium might modify the salt permeability of the membranes, the conclusion that the effect is to neutralize the electroosmotic component may be too simple. Obtained by the same method, the results of Studener (75) do not necessarily support the theory of Brauner & Hasman, since solutions of calcium nitrate and of potassium nitrate as well, often had the opposite effect of increasing the DPD of *Rhoeo*, onion, and *Nitella* cells over that measured in sugar solution. However, in amounts of 0.01 and 0.05 M added to glucose solutions, nitrates of calcium, potassium, and sodium all reduced the DPD in the neighborhood of 1 atm. One might expect different responses from calcium nitrate and potassium nitrate [cf. Steward, Stout & Preston, (54)].

Reinders (53) had previously found that calcium reduces the water-absorbing capacity of potato tissue, but she does not favor the electroosmotic explanation because electroosmosis is known to be greatest in pure water, yet there were no significant differences between water intake from distilled water and from 0.01 M $CaCl_2$. Dilute potassium chloride solutions as a rule were found to increase water absorption.

Although electroosmosis probably occurs in many plant tissues it is doubtful if it is of much significance in the total water relations. According to van Overbeek (57), Blinks regards electroosmosis in roots as unlikely to cause root pressure because current flow is too low and the high salt concentration unfavorable for electrokinetic phenomena. Lundegårdh (79) studied electrical potentials in wheat roots and never found a difference in potential between surface and interior of more than 100 mv. While even a small difference might cause some water movement he calculated that a potential difference of 150,000 mv. would be required to raise water 1 m. Blinks (78) measured a potential difference of 70 to 80 mv. across the cell membranes of *Halicystis*.

While Brauner & Hasman (74) ascribed only 10 per cent of the total forces responsible for water intake by potato tissue to electroosmosis, they later claimed (59) to have demonstrated an anomalous component for turgid carrot root parenchyma which had been in aerated distilled water 18 hr., in the amount of 4.0 to 4.5 atm. This high value may be subjected to the thermodynamic analysis proposed by Levitt (47). The energy necessary to maintain water intake against a diffusion pressure gradient by electroosmosis would be the same as that calculated for any other mechanism, and thus electroosmotic components of this magnitude might be questioned. Few experimental data concerning water movement against a diffusion gradient are available. Frog skin is said to cause water movement against a hydrostatic pressure gradient of only two to four inches (80). Brauner (81) investigated rates of nonosmotic water movement across artificial membranes, but gave no data concerning the pressure developed.

In an impressive attempt to explain absorption, secretion, and the transport of liquid in the plant, Arens (117) presents a picture of what he terms the "active membrane." The tonoplast is considered the prinicpal active membrane of the cell, electroosmosis is the basic process which it controls, and oxygen gradients and respiration account for the energy supply. "Concentrically polarized" cells are capable of primary secretion and function, according to Bennet-Clark's original hypothesis. "Laterally polarized" cells absorb at one end and secrete at the other, due to a difference in oxygen pressure; this Arens calls secondary secretion.

Other evidence.—It is conceivable that some other kind of process or condition may be present in plant cells which supplements osmotic pressure in bringing water into the cell. Secretory forces might result from local changes in osmotic pressure. This concept, originating with Pfeffer (82) and supported by Blackman (83) has been experimentally realized in *Nitella* cells [Osterhout (84)], and is supported on theoretical grounds [Franck & Mayer (85)]. It remains to be demonstrated whether such a process operates naturally in living cells.

Lyon (86) reported that the DPD of potato tissue determined directly by the change in volume method was considerably lower than the DPD calculated by means of the minimum cell volume method. The latter takes into account the estimation of the osmotic quantities (DPD, OP, TP) from measurement of the osmotic pressure at limiting plasmolysis, and volume changes in the three states: limiting plasmolysis, normal state, and full turgor. The data presented lend themselves to various interpretations; for example Levitt (47) finds that by solving for wall pressure the nonosmotic force would be acting in the wrong direction, tending to move water out of the cell. The observed value, i.e. that equal to the OP of a sugar solution causing no change in volume, is probably the correct one. The calculated values are subject to errors of method which involve (*a*) the lack of a critical point at which the tissue is supposed to stop shrinking (limiting plasmolysis), (*b*) the rather high nonsolvent volume of potato tuber cells, and (*c*) the necessity of assuming a straight line relationship between volume and turgor pressure. Ursprung & Blum (87), in a detailed analysis, also deny that this data yields evidence of a nonosmotic force. Notwithstanding, there must be an explanation for the different behavior of the tubers which Lyon observed at different seasons of the year.

The water relations of guard cells are far from being completely understood. Wilson (88) has demonstrated an apparent lack of connection between photosynthesis and guard cell movement in several plants and believes that no existing hypothesis adequately explains all of the observed facts. On the other hand Heath (89) reports an extreme sensitivity of guard cells of *Pelargonium* and wheat to carbon dioxide concentration, which he suggests favors the well known hypothesis that removal of carbon dioxide by photosynthesis accounts for stomatal opening and accumulation of carbon dioxide in darkness causes closure. Liebig (90) noted only insignificant changes in the osmotic pressure of guard cells of *Tradescantia viridis* between the opened

and closed conditions. Failure of the guard cells to function in nitrogen-deficient plants [Gessner & Schumann-Petersen (91)], and following treatment with β-naphthoxyacetic acid [Ferri & Lex (92)] are recent observations that will have to be accounted for in any complete explanation of guard cell mechanism. Assuming that turgor changes are responsible for stomatal function, we would like to know to what these changes are due. It is not our suggestion that there is an active water absorption mechanism here; but it does seem that a simple osmotic explanation is impossible.

The suggestion of Lewis (93) that mesophyll cells "secrete" water is based upon his observation that water globules formed in the intercellular spaces of leaf sections mounted in paraffin oil and viewed with strong transmitted light. It is, however, possible that permeability changes or other effects of reduced oxygen supply or strong light could produce this result, and, as a matter of fact, many other kinds of tissues behave in this way.

In the animal realm there apparently is evidence for active water transport by the kidney tubule and intestinal wall (94, p. 623). Interesting also is a paper by Lees (95) presenting evidence for secretion by the epidermal cells of ticks.

Active versus passive absorption.—It is clear that insufficient evidence is available to completely evaluate the relative importance of active and passive absorption mechanisms. There seems to be no doubt that water intake by plant cells is affected by their metabolic activity, but this may be partially exerted through effects on permeability, on solute concentration, or in other ways. On the whole it seems probable that most of the water exchange is an osmotic or passive process, and that active absorption in which water is moved by direct expenditure of metabolic energy is at least quantitatively less important. Under some conditions, however, it is conceivable that a slight contribution by active forces toward water uptake might be the critical factor in the over-all process. Attention will now be given to other aspects of cell water relations which are important regardless of the exact mechanism causing water movement.

PERMEABILITY OF CELL MEMBRANES TO WATER

The early concept of cell permeability as a static condition analogous to the behavior of membranes in artificial osmometers was gradually abandoned as it began to be realized that permeability of protoplasmic membranes is a variable condition, related to metabolic activity of the protoplasm itself. The differential permeability of cells is largely attributable to the two surface membranes. One, the ectoplast, forms the outer layer next to the cell wall, the other, the tonoplast, is in contact with the vacuolar sap. According to one view these plasma membranes consist of films of lipoid material adsorbed to which is a protein layer, forming a heterogeneous sieve or pore effect. According to another view the surface consists of a lipoid-protein mosaic (94, 119). In any event it is a complex, heterogeneous system containing lipids, proteins, and water, and its organized structure is dependent on metabolic activity.

Although the protoplasmic membranes are relatively impermeable to solutes they are relatively permeable to water. Levitt (47) estimated that parenchyma cells are 1,000 times as permeable to water as to potassium. Permeability of cells to water is most often measured by the rate of plasmolysis or deplasmoysis or by change in volume, though other methods are sometimes used. Levitt, Scarth & Gibbs (96) found water to pass through protoplasts of onion bulb scale at a rate of 0.3 cubic micron per minute per square micron of cell surface when subjected to a DPD difference of 1 atm. Brooks & Brooks (13) cite other values and the existence of a considerable range of values is to be expected since permeability to water may vary from cell to cell and from tissue to tissue. Rosene (97) concluded that different parts of a given cell may vary in permeability to water as she found different rates of water intake through different parts of the same root cell. Permeability varies with time and is involved in endogeneous rhythms manifested by plants [Bünning (32)]. It is affected and controlled by several known factors. Water permeability is greater in meristematic and senescent cells than in those of intermediate age [Maximov & Mozhaeva (98)] and according to Levitt & Scarth (99) cold-hardened tissue is more permeable to water than unhardened tissue.

Numerous studies of the effects of salts have been published. The effect of calcium in decreasing and potassium in increasing water absorption by tissues was discussed under *Electroosmosis;* whether this has anything to do with permeability is uncertain. Brauner & Brauner (100) suggest that water movement through cell membranes depends on two factors. One is an electrical effect depending on the electrokinetic potential of the membranes and the other is a mechanical filter effect. Permeability is greatest near the isoelectric point of the membrane where hydration and viscosity are minimal. In certain membranes such as calf's bladder and onion bulb scale, however, permeability increases as hydration increases. Homès (101) found that permeability of cells of *Dahlia* root increases with increased hydration. This also was the conclusion of de Haan (102) for onion epidermis. Griep (103) believed that permeability is reduced in highly hydrated cell membranes because they are so much swollen that intermicellar spaces are reduced in diameter. Dehydration results in shrinkage, also reducing intermicellar spaces and permeability. Aykin (104) observed that rate of outward movement of water decreased as the concentration of the external solution was increased and reabsorption of water was slowest in those pieces of tissue soaked in the most concentrated solutions, apparently because of greater dehydration of the membranes. Levitt, Scarth & Gibbs (96) found that permeability, as measured by rate of entrance of water, increases as deplasmolysis occurs. This increase in permeability is regarded primarily as a mechanical effect of extension of the membranes. The investigators claim that it is not the result of increased hydration because decrease in permeability was not observed during plasmolysis.

Gaumann & Jaag (105) reported that *Fusarium lycopersici* produces a toxic substance, lycomarasmin, which causes an irreversible change in per-

meability of the cells. As a result of the loss of cell contents, affected tomato plants wilt, even in a saturated atmosphere. Thus, the wilting of fusarium-infected tomato plants is not caused by lack of water, but by loss of differential permeability of the cell membranes. Yarwood (106) showed a similar increased permeability of bean leaves infected with powdery mildew and rust. Brauner & Brauner (107) found that light slightly increased the permeability of carrot root tissue and Weber (108) observed a similar situation in leaves of *Ranunculus ficaria*. Lepeschkin (109) has repeatedly stated that light increases permeability of protoplasm to solutes, proably because it destroys unstable lipo-protein complexes in the protoplasmic membranes.

Brauner (110) reported polarity effects in certain membranes, in the sense that water moved about 50 per cent faster from outside to inside than from inside to outside through *Aesculus* testa. Since this difference in permeability was eliminated by adding potassium sulphate it was attributed to electroosmotic effects. More recently Hasman (111) ascribed a similar polar water movement in potato tissue to a greater flow resistance inward than outward. In another paper Brauner (112) reported that increasing the osmotic pressure of the solution on one side of the membrane from 14.6 to 58.4 atm. only increased the rate of diffusion across the membrane by 50 per cent. Increasing the pressure across the membrane from 0.46 to 0.92 atm. with a vacuum produced less change in rate of water movement than increasing it with a pressure pump. Kramer (113) found that water movement across a multicellular plant membrane along an osmotic gradient of 12 atm. was much slower than water movement under a pressure or suction gradient of 1 or 2 atm. Application of pressure to the outside of this membrane caused much faster water movement across the membrane than application of suction to the inside of the membrane. This curious difference in behavior was not explained.

Little attention has been given to the relative permeability of the wall versus the cytoplasm. Skene (114) claimed that the protoplasm offers 50,000 to 80,000 times as much resistance to the movement of sucrose as does the cell wall. Levitt, Scarth & Gibbs (96) claim that the permeability of free protoplasts is about the same as that of protoplasts enclosed in their walls, indicating that the wall is relatively much more permeable to water than the cytoplasm. This view is supported by Kramer's (115) observation that there is a large increase in water movement through roots subjected to a fixed pressure gradient after they are killed. Dead roots also are much more permeable to water at low temperatures than living roots, indicating that most of the reduction in permeability caused by cooling occurs in the cytoplasm rather than the wall [Kramer (116)].

In conclusion it should be emphasized that permeability of protoplasmic membranes to water, as to solutes, is in some way related to the metabolic activities of the cell. The exact relations are still unknown, but since protoplasmic membranes are exceedingly complex in structure a certain amount of energy is required to maintain the structure. Fluctuations in energy supply doubtless result in changes in fine details of the protoplasmic structure,

resulting in changes in permeability. Some energy may also be expended in maintaining differences in electrical potential.

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SOIL MOISTURE IN RELATION TO
PLANT GROWTH

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This report is a review of plant growth responses to soil moisture. It does not include the voluminous literature dealing with the many other aspects of soil moisture. No final decision regarding the responses of plants to soil moisture can be reached from purely theoretical considerations, but must be determined with plants in the field. Therefore, in this review, more attention has been paid to actual trials with plants than to papers dealing principally with the theoretical considerations of the problem.

Responses of plants to soil-moisture conditions are generally well known to farmers in irrigated areas. Among these are the vigorous growth of plants that are not allowed to suffer from lack of water, the various symptoms shown by different plants when subjected to dry soil conditions, and the rapid recovery and resumption of growth when water is supplied to the soil following a dry spell.

The concept of the soil as a reservoir for water is probably more clearly recognized in irrigated sections than in areas having frequent summer rains, because of the necessity of replenishing the supply at intervals. In areas where rainfall occurs during the growing season, this concept is perhaps not so widely recognized, because the reservoir is replenished by rain and is generally filled or partly so, except when unusually long rainless periods occur.

The upper and lower limits of water storage in the soil reservoir are fixed by two soil-moisture conditions, which might be called soil-moisture constants. In fact, the authors believe they are the only ones of any practical value for consideration in connection with plant growth. The first of these, the field capacity, is the amount of water held in a soil after excess water has drained away and the rate of downward movement has materially decreased. This usually takes place within two or three days after rain or irrigation in pervious soils of uniform structure and texture. Below the first foot and in the absence of vegetation the field capacity persists for months without much change.

The lower limit of the soil reservoir or the moisture content at which we may consider it to be emptied, since it no longer contains sufficient water to maintain normal growth and vigor of plants, is the permanent wilting percentage. As the authors have pointed out (1948b), this moisture content is so important that special consideration should be given to it. The most important early work on the availability of water to plants is that of Briggs & Shantz (1912) who studied 20 soils and made some 1,300 trials. They concluded that on a given soil all plants reduce the moisture content of the soil to about the same extent when permanent wilting is attained, a condition of

wilting such that recovery does not take place until water is added to the soil. The residual moisture in the soil at the time plants wilt permanently, therefore, is a soil characteristic.

Brown (1912), Caldwell (1913) and Shive & Livingston (1914) disagreed with the Briggs & Shantz concept and stated that permanent wilting is determined by climatic and not by soil-moisture conditions. The authors (1934, 1945) found that the residual soil-moisture content at permanent wilting is remarkably constant for a given soil under any evaporating conditions likely to be obtained with plants growing in the field. Furr & Reeve (1945) report similar results.

Briggs & Shantz' conclusion that all plants wilt at the same moisture content when grown on the same soil has been questioned by a number of investigators. Lane & McComb (1948) believe that the permanent wilting percentage is lower for grass than for trees when grown in pots. Fowells & Kirk (1945) state that pine seedlings reduced the moisture content of a soil to a lower percentage than sunflowers. Koketsu (1926) believes that the residual soil moisture at the time of permanent wilting is not constant for a given soil but is affected by the nature of the plant. The authors have tested many plants by growing them in the same kind of soils in small containers and also in field trials. The results substantiate the conclusion of Briggs & Shantz, which now seems to be accepted by most investigators.

Briggs & Shantz (1912) reported results which seemed to show that the residual soil moisture at permanent wilting, which they term the "wilting coefficient," bore a fixed relation to other soil properties, such as hygroscopic coefficient, water-holding capacity, mechanical analysis, and moisture equivalent. The latter is the one most frequently used since it is a simple laboratory determination. The wilting coefficient was believed to bear a constant relation to it. In fact, the term "wilting coefficient" may have been suggested because it was thought that dividing the moisture equivalent by the factor 1.84 would give the residual moisture in the soil at the time plants would permanently wilt. It has been pointed out by the authors (1928), Duncan (1939), Shaw & Swezey (1937), Wadsworth (1929), and others that this ratio does not hold for all soils. The constant relation of the wilting coefficient to other moisture properties, as Briggs & Shantz suggested, has been found by the authors (1934) not to hold for all soils. Schofield & Botelho Da Costa (1935) reported a soil with a ratio of 8. Wadsworth (1934) found that some of the Hawaiian soils have ratios as low as 1.11.

The authors (1929) proposed the term "permanent wilting percentage" in place of "wilting coefficient," since the latter infers a definite relation to other soil properties which, as now known, does not hold for all soils. If the term "wilting coefficient" is used it should be clearly stated whether the value is calculated from some other measured one or actually determined by wilting plants. Some prefer to shorten the term "permanent wilting percentage" to "wilting percentage" or "wilting point."

Plants growing in cans or in the field continue to use some water after

they are wilted. After wilting permanently, the soil continues to lose moisture. It is well known that all the leaves on a plant do not wilt at the same time and the question then arises as to what part or parts of the plant should be used as a criterion of wilting. It is impossible to tell whether a plant has gone beyond permanent wilting. If it has not reached the permanent wilting percentage, recovery takes place in the moisture chamber. The plant which has just permanently wilted is essentially no different in appearance from one which has been wilted several hours, but the soil-moisture content may have changed.

The reduction of soil moisture with plants in containers after the plants have wilted is in agreement with the work of Taylor, Blaney & McLaughlin (1934). They propose the term "ultimate wilting point" to designate the soil-moisture content when all the leaves have wilted and define the wilting range as the range in moisture content of the soil between the wilting coefficient and the ultimate wilting point. Furr & Reeve (1945) use the term "first permanent wilting point" to indicate soil-moisture content at the time the basal leaves of sunflowers wilt permanently. The range of moisture between this moisture content and the ultimate wilting point is called by them the "wilting range."

The authors (1945) have shown that plants with a single leaf or with a pair of opposite leaves can be used to determine the permanent wilting percentage, the results agreeing with those obtained with entire plants. Furthermore, the moisture-extraction curves from field samples show only a slight reduction after the permanent wilting percentage is reached. This reduction is small, often not more than 1 per cent, even after three or four months. These extraction curves practically coincide year after year, both as to slope and as to minimum moisture content reached.

Briggs & Shantz (1912) showed nearly horizontal curves after the plants wilted. Burr (1914) showed with wheat that the soil-moisture extraction curves were substantially horizontal in the latter part of the growing season. On the other hand, Taylor & Furr (1937) give curves for citrus from which they concluded there is a downward slope approximately the same as that obtained from sunflowers in cans. Esselen (1937), also working with citrus fruits, showed that the extraction curves for unirrigated portions of the orchard were horizontal, or nearly so, for about six months. Mathews & Chilcott (1923) and Cole & Mathews (1939) showed that after the readily available moisture is exhausted reduction of soil moisture is very slow and generally is within the range of experimental error. Swezey (1942) found that sugar cane plants growing in the field reduce the soil moisture appreciably below the permanent wilting percentage. The authors (1945) believe that the wilting range is much narrower for plants in the field than for those in containers. They define the permanent wilting percentage not as a unique value but as a small range of soil-moisture contents within which permanent wilting takes place. This range need not exceed 1 per cent for fine textured soils or 0.5 per cent for coarse textured ones.

PHYSICAL MEASUREMENTS OF SOIL-MOISTURE CONSTANTS

Indirect methods of measuring the field capacity and permanent wilting percentage have been proposed. The former, usually in the absence of field measurements, is determined in the laboratory by the moisture equivalent. This value was first suggested by Briggs & McLane (1907) and later modified by Briggs & Shantz (1912). Tests which indicate that the moisture equivalent is a close approximation of the field capacity have been reported by Burr & Russel (1925), Mathews & Chilcott (1923), Piper (1933), Scofield & Wright (1928), and the authors (1931). But below 12 to 14 per cent, the moisture equivalent seems to be less than the field capacity. It is a fair measure of the field capacity of fine textured soils, but not necessarily of the sands. Richards & Weaver (1944) found that the moisture retained by samples placed on a suction plate at a tension of $\frac{1}{3}$ atm. corresponds closely to the moisture equivalent. Smith (1944) and the authors (1946a) point out that moisture equivalents made on fragmented samples taken from the field may not agree with the field capacities of the soil in place in the field.

Various methods have been proposed to measure the energy necessary to remove water from soil. Terms for it, such as "free energy," Edlefsen & Anderson (1943), "pF," Schofield (1935), "capillary potential," Buckingham (1907), "moisture potential," Day (1942) and Veihmeyer & Edlefsen (1937), "thermodynamic potential," Gardner (1944), and "total potential" and "soil moisture stress," Wadleigh (1946a) have been suggested. Whether the potential is a continuous function of soil moisture or a multiple-valued one has been discussed by Haines (1930), Smith (1944), Veihmeyer & Edlefsen (1937), Edlefsen (1933), and Wadsworth (1944). Edlefsen & Smith (1944) point out that the difference between a real hysteresis or multiple value and a lag in equilibrium must be clearly distinguished and, further, that hysteresis under field conditions is of little importance.

So far as can be judged from existing data, the potential of the soil moisture at the permanent wilting percentage is the same for all soil, although this may be open to question due to limitations of the methods now available to determine it. Woodruff (1940) believes that it is not safe to assume that the moisture content of a soil will be constant for a given pF value unless the sample of soil is handled in a specified manner. Vapor pressure measurements by Edlefsen (1934) and Thomas (1921), freezing point depression by Schofield (1935), and dilatometer measurements by Anderson & Edlefsen (1942) and Bouyoucos (1936) indicate that about 16×10^8 ergs per gm. are required to remove unit mass of water from a soil at the permanent wilting percentage. Bodman & Day (1943) believe that the energy of retention at the permanent wilting percentage is approximately 18×10^8 ergs per gm. Richards & Weaver (1944), by means of the pressure membrane apparatus, have taken the value as equivalent to that required to remove water from a 15 atm. solution.

The authors (1934) point out that the magnitude of the resistance to removal of water at the permanent wilting percentage is much greater than

that required for removal from a 4 atm. solution as suggested by Shull (1916), following his studies to determine the amount of water that calibrated seeds would absorb at different soil-moisture contents. They show that plants grown in solution containing nontoxic solutions, such as sucrose, do not wilt until the osmotic concentration reaches a value of about 16 atm.

Typical vapor pressure curves for soils show the small decrease in vapor pressure from the field capacity to the permanent wilting percentage and the very rapid decrease thereafter. The position of the permanent wilting percentages on these curves near the region where the tightness with which the water is held by the soil increases very rapidly is significant.

This fact strengthens the selection of the permanent wilting percentage as the most important soil-moisture constant in consideration of plant-soil-moisture relations. It also lends support to the conclusion that above the permanent wilting percentage soil moisture is readily available to plants. The authors (1948b) have used the term "readily available water" to mean that water between field capacity and the permanent wilting percentage. Some water must be available to plants even though the soil is at the permanent wilting percentage and there must be some extraction of water below this moisture content; therefore, the soil-moisture extraction curves would show a slight downward slope. They have shown (1945, 1948b), however, that the reduction of moisture in field soils below the permanent wilting percentage is very slight below the surface layer affected by evaporation.

Attempts have been made to change the permanent wilting percentage in hopes of providing a larger amount of readily available water by the addition of organic matter and fertilizers. Alway & Neller (1919), Feustel & Byers (1936), Proebsting (1937), and Veihmeyer (1938) have shown that organic additions to soils, in some cases greatly in excess of that possible in commercial practice, have not increased the amount of readily available water in soils. The application of large quantities of soluble material to soils may change the amount of readily available water as Wadleigh (1946b), Magistad (1945), and their colleagues have pointed out. Failure of plants to grow satisfactorily also may be due to the toxic effect of the added material, as well as to the increased osmotic value of the soil solution.

The Availability of Water to Plants

The conclusion that there is an end point below which plants do not obtain water fast enough nor easily enough to maintain normal growth has general acceptance. There are, however, wide differences of opinion and much controversy as to the availability of water between the field capacity and the permanent wilting percentage. On the one hand, it is held that water is equally available to plants throughout the range from the field capacity to the permanent wilting percentage; on the other, that plants respond favorably to high soil-moisture conditions and that adverse effects will result as the water content decreases. It is not very helpful to say that there is abundant evidence to support either contention. Each piece of work requires

careful scrutiny of the data which are used to support the conclusions reached.

Most of the work has been done by growing plants in small containers and most of the opinions concerning the influence of moisture on plant growth have been obtained from them. A fewer number of experiments have been carried on in the field. The authors believe that container experiments may be valuable to indicate trends or to measure what might be called end points such as the permanent wilting percentage, but certainly they should not be taken as being conclusive until they are verified by field trials. One discrepancy has already been noted, i.e. the difference in extraction of moisture in containers below the permanent wilting percentage and that which actually occurs in the field. A further example of the lack of agreement between water use by plants in containers and in the field is given in a report by Kittredge (1941).

TRANSPIRATION AND PHOTOSYNTHESIS IN RELATION TO SOIL MOISTURE

The evidence that the rate of transpiration is affected by variations in moisture content within the range from field capacity to permanent wilting percentage is conflicting. The uniformity of the slope of the moisture-extraction curve with plants in containers indicates that moisture is equally available for transpiration between the field capacity and the permanent wilting percentage. Furr & Taylor (1939), Veihmeyer (1927), the authors (1945), Wadsworth (1934), Shaw & Swezey (1937), and Kokin (1925) have reported similar results.

Additional studies indicating the equal availability of soil moisture down to the permanent wilting percentage include: Crowther (1934), who found the net assimilation rate to be unaffected by water; Hendrickson (1926), who showed that soil moisture can fluctuate through wide limits without affecting the width of stomatal opening or the moisture content of leaves, twigs, wood, and bark; Finch & Van Horn (1936), who noted that the per cent moisture in mature leaves of pecan growing under orchard conditions to be nearly constant regardless of differences in soil moisture when conditions for maximum transpiration obtain; Caldwell (1913), who reported that the water content of turgid leaves of four different plants never rose very high above the point at which temporary wilting occurs; Koketsu (1928), who noted that the water contents at full turgidity, slight wilting, and at permanent wilting are probably constant; Burns (1926), who found the photosynthetic activity of forest trees to be independent of soil moisture down to the point where death occurred for lack of water; and Overley, Overholser & Haut (1931), who observed no variation in stomatal response of apples between the wilting point and field capacity.

Schneider & Childers (1941) and Loustalot (1945) measured transpiration and photosynthesis of apples and pecan leaves by passing air through a dehydrating agent after passing over the surface of a leaf enclosed in a chamber

and determining the increase in weight of the agent due to the water absorbed. They concluded that transpiration and photosynthesis were affected by changes in amounts of readily available soil moisture.

The fact that only a few leaves on the trees were measured each time makes it doubtful whether they represented the real transpiration of the entire leaf surface, since all leaves may not have been transpiring at the same rate. The enclosure of the leaves in a water-tight chamber which may have changed the transpiration rate casts further doubt on the method used. No soil-moisture records were reported by Schneider & Childers; only the dates following irrigation are listed. It is interesting to note, in their experiment II, page 571, that a period of about eight days elapsed before the test plant wilted, but for the first six days of this period the transpiration they reported was the same for both check and test plant. In their experiment III, again a period of about eight days elapsed between waterings, but there appears to be little difference in transpiration until the last two days of the period. These records indicate no difference in transpiration and photosynthesis through a considerable range of soil moisture, but how close to the permanent wilting percentage it was reduced is not evident. Loustalot's data, reported in the form of graphs, permit some analysis. The seedling pecans on sand show rates of photosynthesis and transpiration substantially constant until just before the soil moisture is reduced to the wilting coefficient which is given as 1 per cent. It is not known whether it is a calculated or measured value. The rates were constant throughout a range of soil moisture from 6 to 2 per cent. The seedlings on soil show constant rates of photosynthesis and transpiration throughout a range of soil moisture from about 30 per cent to very close to the wilting coefficient which is given as 12.2 per cent.

In carefully conducted experiments Martin (1940) grew sunflower plants in pots and concluded that the rate of transpiration per unit of leaf surface was ordinarily affected when about two-thirds of the available water had been removed. Since in all cases in which the transpiration rate and stomatal opening were affected, the leaves showed signs of wilting, it would appear that the soil moisture must have been reduced close to the permanent wilting percentage.

Hartt (1936) studied photosynthesis of sugar cane plants grown in small pots which seem to be very small in relation to the size of the plants. She showed that the synthesis of carbohydrates takes place in the blades of plants which are at or below the wilting point, but synthesis is greater in blades of the watered ones. Her data do not show the relation between synthesis and the variation in amounts of readily available water. Variations in soil moisture found by Furr & Degman (1931) have a measurable though slight influence on fruit growth and a marked influence on stomatal behavior, even when the soil moisture is several per cent above the wilting percentage.

Carbon dioxide intake is stated by Allmendinger, Kenworthy & Overholser (1943) to be uniform throughout a wide range of soil moisture from about 37 per cent to approximately 10 per cent. They report the wilting

point of their soil to be 7 per cent. The difference in average intake of the gas for the entire period of observation does not seem to be significant for the three trees in which all the readily available water was utilized and for those of the other treatments.

Kramer (1944) believes that his own work on exudation from the stumps of detopped plants indicates that water is not readily available to plants throughout the entire range from field capacity to permanent wilting percentage. Such a system, of course, is not comparable to an entire plant, since water absorption in a detopped plant is due to the so-called active suction pressure (or osmotic forces), while in the whole plant forces of much greater magnitude, due to the transpiration pull, are involved in the absorption of water. Kramer states that exudation ceases when about 45 per cent of the available water is still present. Using the same technique, McDermott (1945) believes that a total of 60 per cent of the available soil moisture is not available for exudation. McDermott has fitted parabolic curves to his data. With the possible exception of the results for his series B experiment, horizontal lines might be a better fit for the data. His series A and C results do not justify a parabolic curve. Except for about four measurements in series D and two in series E, the same may be said for them. It is true that in his series B there is a group of measurements which shows high negative exudation for the lowest moisture content, but the extreme variability of the measurements at about 10 per cent soil moisture raises a question whether the extremely low group should be given much weight in determining the shape of the curve.

<h3 style="text-align:center">MEASUREMENTS OF PLANT GROWTH RATES IN RELATION TO SOIL MOISTURE IN POT EXPERIMENTS</h3>

Normal growth over the range of soil moisture from the field capacity down to or close to the wilting percentage has been reported by Fowler & Lipman (1917) working with lemon trees in small tanks, Taylor & Furr (1936) who studied potted lemon trees, Wadsworth (1934) who grew sugar cane in tanks, Shaw (1930), Wadsworth (1932, 1941), and Shaw & Swezey (1937) in similar work on sugar cane, Daubenmire & Charter (1942) in studies on woody desert legumes, Went (1944) working with tomato, and Anderson & Kerr (1943) studying cotton boll enlargement. Furr & Reeve (1945), as a result of experiments with sunflowers in small cans, could come to no definite conclusions as to the relation between growth rate and soil moisture above the wilting range and Beach (1939) reported that although carnations growing in greenhouse benches appeared to be superior when kept under wet soil conditions as compared to medium and dry, the yield and quantity tended in the other direction.

In contrast to these reports are a number of pot experiments indicating interference with growth by soil moisture levels above the permanent wilting percentage. Cykler (1946) grew potatoes in tanks and concluded that top growth is independent of the moisture content as long as available moisture

is present, but that high yields can be obtained by keeping the soil moisture at a high level and not letting it fall below one half the range between field capacity and permanent wilting percentage. He believed that the rate of extraction of moisture was constant for all treatments. In a later paper (1947) he concluded from field trials that the moisture per cent should not be reduced below two thirds of the available range. Davis (1940) thinks corn plants in pots cease to grow when the soil moisture is 3 per cent above the permanent wilting percentage. He also concludes (1942) that the growth of nut grass in pots is checked when the soil moisture is reduced. From studies of corn plants in jars Haynes (1948) concluded that growth was affected by the degree of availability of soil moisture from near saturation to the permanent wilting percentage, but that transpiration was not affected. The small size of the jars in relation to the size of plants may be an important factor in his study.

Ayers, Wadleigh & Magistad (1943) and Wadleigh & Ayers (1945), from studies with beans in 10-gallon cans, showed that plant growth was inhibited as the soil moisture tension at the time of irrigation increased, even though in some of the treatments the soil moisture was always above the permanent wilting percentage. The soil moisture in the high tension series without salt treatment was at the permanent wilting percentage and the yields were low, but the difference in yield between the low tension (high soil moisture) and medium tension treatments may be of questionable significance. These investigators concluded that the hyperbolic nature of the relationship between soil moisture percentage and moisture tension accounts for the frequent finding that for all practical purposes plants may not show changes in growth responses while reducing the moisture percentage of soil from field capacity to nearly the wilting percentage.

Wadleigh & Gauch (1948) concluded from a study of cotton plants in 100-pound drums that leaf elongation ceased at a moisture stress close to 15 atm. and stated that elongation was expressed as a second degree function of soil moisture stress. It is interesting to note that leaf elongation shown in their figure 5 was approximately at a uniform rate until the stress was increased to about 13 atm. in the first cycle of irrigation.

MEASUREMENT OF PLANT GROWTH RATES IN RELATION TO SOIL MOISTURE IN FIELD EXPERIMENTS

Experiments with deciduous fruits.—The work most frequently cited in recent years in support of the belief that growth of plants in the field shows that water is not equally available over the entire range of readily available moisture is that of Aldrich & Work (1932, 1934), and Lewis, Work & Aldrich (1934, 1935), all of which was done in a pear orchard on very heavy clay soil. The statement by Work & Lewis (1936) that the soil in the orchard from which these data were gathered was so heavy that root distribution was very sparse, is often overlooked. They stated that even though the soil in contact with the roots may be dry, at a little distance away it may be moist. The

samples taken to determine moisture content included dry and wet soil, giving an average above the permanent wilting percentage. Lewis, Work & Aldrich (1935) explain that the combination of slow moisture movement of water through the tight clay soil in their orchard and the relatively sparse root population in the soil accounts for the effect on growth of comparatively small changes in the average moisture content of the soil in the root zone. Obviously, the results of their experiments cannot be taken as a contradiction of the authors' statement (1929) "that trees either have readily available moisture or have not," because these investigators did not know the moisture content of the soil in contact with the absorbing portion of the tree roots. The authors (1948a), furthermore, have shown that the Meyers clay adobe in this orchard would not permit the entrance of roots with an apparent specific gravity of 1.52, a density very near that in the field. Much of the root development must have been confined to the soil cracks. It is, indeed, surprising that this work has been so widely quoted without regard to the limitations introduced by the character of the soil.

Magness, Degman & Furr (1935) studied the effect of irrigation on the growth of apples in Eastern orchards and concluded that apple trees are not measurably reduced in function, and growth rate of fruit is not measurably reduced so long as the content of practically the whole root zone is above the wilting percentage. Boynton & Savage (1938) report the seasonal fluctuation of soil moisture in apple orchards and even though the soil moisture varied from field capacity to permanent wilting percentage in some of the orchards, they concluded that lack of soil moisture probably seldom limits the productivity of New York orchards on well drained soils which permit rooting to a depth of 4 feet. From their studies with apples in Eastern orchards, Furr & Magness (1930) concluded that "trees can function at near the maximum rate so long as the moisture content of the whole root zone is appreciably above the wilting percentage." Harley & Masure (1932) found that apples on sandy and clay loam soils showed no significant decrease in volume growth when the soil-moisture content fell as compared to fruit grown with an abundance of water. They state that varying percentage of water is of minor importance in plant growth, as long as the whole root zone remains above the wilting percentage.

Following results obtained with apples in Maryland, Furr & Degman (1931) believe that relative amounts of available soil moisture had a measurable though slight influence on fruit growth and a marked influence on stomatal behavior while the soil moisture was several per cent above the wilting percentage. The fruit growth of the Grimes apples, however, was the same whether irrigated or not. That of the Delicious variety in the non-irrigated plots was only slightly different from the irrigated. Overley, Overholser & Haut (1931), from studies with apples in Washington, state that the average size and color of the harvested fruit from the various plots showed considerable variation, which was probably due to a difference in load of fruit per tree or the leaf area per apple rather than to the spray or irrigation

program. Cullinan & Weinberger (1932) report studies on peaches in Maryland and show there is little difference in the growth of the fruit until after the soil moisture in the dry plot was reduced to the permanent wilting percentage, at which time the fruit in the normal treatment shows greater growth.

The authors' (1942, 1946b, 1948c) results with pears, prunes, and apples in California show that growth of fruit is not affected by changes in soil moisture unless it is reduced to the permanent wilting percentage. Keeping the soil moisture high in the available range by irrigating before the readily available water was exhausted did not increase the size of pears or apples. The authors also showed (1929) that the rates of growth of peaches and the growth of Muir peach trees were not affected unless the soil moisture reached the permanent wilting percentage. Mature deciduous fruit trees make one flush of growth and then stop, whether the soil moisture is kept high in the available range or allowed to decrease. Furthermore, irrigation, after the terminal buds have formed, does not promote additional growth. Some young fruit trees, however, may make more than one flush of growth on a diminishing soil-moisture content without the application of additional water. These facts must be taken into account in evaluating the results of measurements of tree responses to different soil-moisture conditions.

Experiments with dates.—Moore & Aldrich (1938) studied the growth of dates in relation to soil moisture. The reduced rate of growth of the dates in their plot A as compared to that of the irrigated plots B and C occurred about the time the soil reached the permanent wilting percentage to the depth recorded.

Further studies on the effect of soil-moisture deficiency on dates have been made by Aldrich, Crawford & Moore (1946). The record of daily elongation of leaves of the palms indicates that the rates were the same for all treatments until about the middle of June. The soil-moisture content in the B plot was reduced to the wilting range in the soil below the 2-foot depth shortly after June 1. And in the top two feet at the time there appeared to be a difference in growth rate between the trees in this plot and those in A plot which were frequently irrigated and, for much of the time, the moisture content was above the moisture equivalent. The leaf elongation in B plot was again reduced when the soil moisture below two feet reached the wilting range about the middle of August. The fresh weight per fruit in B plot shows some reduction over that in A plot about the middle of June, coinciding with the time the soil moisture reached the wilting range throughout the full depth sampled, but the final weight of fruit was about the same as that in A plot.

Experiments with citrus.—Beckett, Blaney & Taylor (1930), from results of studies with citrus and avocado trees, concluded that as long as the soil moisture is above the wilting point, the moisture content has no measurable effect on the rate of moisture extraction; that is, moisture is as readily available when the moisture content is one-third or two-thirds of the way be-

tween field capacity and the wilting point as it is in the thoroughly moistened soil after irrigation.

Results of irrigation experiments with citrus trees have been reported by Esselen (1937). He believes that while fruit growth measurements may seem to indicate whether it is necessary to irrigate, the method is not very satisfactory. He points out that there was a rapid increase in fruit size in the plot which had not been irrigated in $1\frac{1}{2}$ months and that the moisture content in the first 3 feet was almost at the wilting point. His curves of fruit growth do show a decrease in rate when the soil moisture is reduced to the wilting point in the space between the tree rows that is irrigated. Esselen agrees with the concept that water is readily available between the field capacity and permanent wilting percentage.

Oppenheimer & Elze (1941), in a report concerning the irrigation of citrus trees in Palestine, do not agree with the results obtained by the authors (1929) with peaches and believe that a decrease in soil moisture undoubtedly exerts physiological effects even above the permanent wilting percentage. These investigators believe there is no clearly defined limit between available and unavailable water. They add that intervals of 36 days between irrigations did not diminish the total seasonal growth of the fruit.

Halma (1934a) showed that Washington navel oranges had practically the same fruit size in two plots, one of which was irrigated after the establishment of a definite water deficit in the leaves while the other was watered before the deficit occurred. In another report on citrus water relations (1934b), he states that fruit growth and soil moisture follow the same general trend, but adds that factors other than soil moisture affect the relative saturation deficit in the leaves to a lesser extent than the fruit. In his report (1934a) Halma says the permanent wilting percentage of the soil is approximately 4.7. If this is true for the portion of the same orchard used in his report (1934b), it is obvious that the soil moisture was reduced to this percentage and stayed there for several days in the dry plot. This may account for the difference in the fruit and leaves reported by Halma. Certainly the soil moisture record in Halma's (1934a) report shows that the growth of trunks of the trees in the dry plot decreases and the increase in relative saturation deficit of the leaves coincides with the time the soil moisture in the dry plot reached the permanent wilting percentage, showing that these plant responses are not affected by variations in amounts of readily available moisture.

Extensive experiments on the growth of lemon fruits in relation to soil moisture were carried out by Furr & Taylor (1939). Their results with two potted lemon trees (page 39), grown under relatively uniform temperature and relative humidity, show that transpiration was constant over a wide range of soil moisture, but decreased when the moisture was about at the wilting range. Likewise, the growth of fruit seemed to be affected only when this same soil-moisture content was reached. In their experiments with trees growing in orchards, these investigators point out the wide variations encountered in field soil sampling and the nonuniform root distribution at

some of the places sampled. They conclude from studies on an orchard on clay loam soil "that the relation between variations in soil moisture and the apparent fruit growth is not very definite. From an examination of the data on soil moisture alone it would be difficult or impossible to determine when the trees suffered water shortage," which they attribute to lack of uniform root distribution. They conclude from their studies on lemon trees on an outwash from a stony sandy loam that "so long as all the soil in the root zone is above the wilting range, variations in moisture percentages above the wilting range have no measurable effect on the apparent growth of fruit."

On a good loam soil they state "that apparent growth rate of fruit was not reduced as a result of decreasing soil-moisture content until the moisture of some of the soil in the top 3 feet reached the wilting range." Again, from their studies on a heavy clay loam soil, they find "that so long as the moisture content of all the soil was above the wilting range the apparent growth rate of fruit was unaffected by variations in soil moisture . . . ." From repeated trials at a later time, Furr & Taylor again concluded that there is a wide range of soil moisture available to the tree and that when appreciable water deficits have been found under orchard conditions, soil-moisture records have shown part of the root zone with moisture contents in the wilting range.

The extended and repeated quotations from the investigations of Furr & Taylor are believed to be justified because of the large amount of careful work they have done, and because, furthermore, reference to their work has been made by others in reviewing the subject of plant-soil-water relations to show that variation in the amount of readily available soil moisture does affect plant growth, a conclusion which is not in accord with that of Furr & Taylor as is evidenced by their quoted statements and the data they present.

Thomas (1922) believed that on the heavy soil with which he worked citrus trees were in better condition when irrigated at sixty-day intervals than those irrigated at thirty-day intervals. Vaile (1924), from a survey of citrus orchard practices, concluded that the intervals between irrigations should be longer in the cooler coastal districts of California.

Jensen (1919) believes that the volumes of lemon fruits are related to the amount of available soil moisture, but there is only one chart in his report where increase in lemon growth is plotted against soil moisture. There was a period from about July 1 to September 1 where the rate of growth of the fruit actually increased even though there was about a week during this time when there was no readily available soil moisture. The validity of his conclusion is doubtful.

Experiments with alfalfa.—The results of the effect on yields of alfalfa by varying the number of applications of water are reported by Beckett & Huberty (1928). They found that on a loam soil, on a nine-year average, variations in the number of irrigations from 3 to 12, when the total seasonal depth of 30 inches was applied, caused only small differences in yields. Even on a sandy soil variations in the number of applications did not materially affect the yield.

These investigations also showed that frequency of irrigation both on the loam and sand did not affect root distribution. Conrad & Veihmeyer (1929) point out that unless adverse conditions for growth are brought about, irrigation can have little influence on the extent of the root system developed.

The effect of frequency of irrigation on the yields of alfalfa on a loam soil at Davis, California, has been studied for a number of years by Doneen (1948). From these unpublished studies, he finds that no higher yields are obtained from the maintenance of moisture high in the range of available moisture, as compared to those where the moisture fluctuates throughout the entire range.

Experiments with cotton.—Martin & Loomis (1923), working with Pima cotton in Arizona, concluded that different frequencies of irrigation after the plants had reached a normal fruiting state did not cause any consistent significant difference in the growth of plants or in the yields. These tests extended from about the first of July to the middle of September. The plants were about 18 inches high and had from five to eight fruiting branches just beginning to flower.

As a result of his studies in New Mexico, Curry (1934) is of the opinion that the cotton plant has a wide adaptability insofar as water is concerned, as high yields were obtained from four or five irrigations, and only slightly higher yields from about twice that number.

McDowell (1934) thinks that with cotton in Texas heavy irrigations applied at longer intervals are better than small applications at more frequent intervals and with abundant moisture in the soil before planting, cotton should not require further irrigation until it begins to show signs of wilting.

The results of cotton irrigation experiments in California from 1926 to 1935 are given by Adams *et al.* (1942). The experiments during the last two years of this period were the most decisive. The results show that the yields were not affected by the irrigation treatments, even though in one of them the moisture was at the permanent wilting percentage in some portions of the soil, but the vegetative growth was higher in the treatments with high soil-moisture contents. This reduction in growth, even though the average soil moisture was not reduced to the permanent wilting percentage, is not in accord with the many results with permanently rooted crops obtained by the group of investigators at Davis, California. The seeming discrepancy may be due to poor root distribution of this plant. King (1922), from his studies with cotton in Arizona, suggests this by his statement

the severe water stress frequently exhibited by plants possessing large areas of leaf surfaces and the shedding of squares and bolls from such plants are not always induced by lack of "available" moisture throughout the soil mass, but may be due to a reduction of moisture in the portion of the soil immediately surrounding the roots more rapidly than it can be restored by capillarity.

Studies by Doneen (1949) show that the sparse root distribution of potatoes and corn does not permit the determination of moisture contents of the soil in contact with the absorbing portions of the roots. Hence, the aver-

age soil moisture, as indicated by sampling the soil, may show it to be above the permanent wilting percentage when actually the soil in contact with the roots may be depleted of its readily available moisture. Similar conditions were found by Veihmeyer & Holland (1949) in studies with lettuce.

Mathews (1948) has reached the same conclusion from his studies with corn in the Great Plains since he finds that the roots of this plant do not occupy the soil completely.

Experiments with sugar cane.—Studies on the response of sugar cane to soil moisture both in tanks and by extensive field experiments by Shaw (1930), Wadsworth (1932, 1934, 1941), Shaw & Swezey (1937), Swezey & Wadsworth (1940), Swezey (1942), and Borden (1948) show that soil moisture may fluctuate throughout the range of readily available moisture without reducing growth of cane or yield of sugar.

Clements (1948) does not agree with this concept. He refers to the statement that plants are able to withdraw soil water with equal facility down to the wilting point and says, "Our studies at Waipio over a 5-year period show this simply not to be true." He states that the field capacity of the soil at Waipio ranged from 35 to 37 per cent. Its wilting point as determined by the sunflower test is said to be 24 to 25 per cent and that whenever the soil moisture dropped below 28 per cent there was a check in growth. This indicates a range in readily available moisture of 11 to 12 per cent, a relatively wide range for Hawaiian soils.

Swezey (1942), also working at Waipio at the same experimental station, gives the field capacity as 36 per cent and first permanent wilting percentage as 28 per cent. He says that the cane plants actually extracted soil moisture 2 to 4 per cent below the permanent wilting percentage and that, except for a reduction in growth rate, which occurred only when the soil moisture was in the wilting range, no visible distress was manifested by the cane during the time this additional 2 to 4 per cent moisture was being extracted. He states,

A reduction of cane tonnage resulted from repeatedly withholding irrigations until some time after the "permanent wilting percentage" was passed, but there was no commercial loss, since the sugar yield was not reduced. . . . It would seem that allowing the soil moisture to fall below the permanent wilting percentage to a still lower limit, at which soil moisture became in fact totally unavailable, was not destructive to the commercial yield from the field.

Experiments carried out at Waialua by Swezey & Wadsworth (1940) show that cane growth is not affected by variations in soil moisture until the wilting percentage is reached. The tons of sugar per acre were not reduced, even though the soil moisture was below the wilting percentage for 150 idle days in its history (one day below the wilting percentage is one idle day).

Experiments with sugar beets and watermelons.—From studies on the irrigation of sugar beets, Brewbaker (1934) suggests that a desirable irrigation practice would consist of early initial spring applications followed by rather frequent light irrigations and a relatively late application in the fall.

Haddock & Kelley (1948) report studies with sugar beets. Without added fertilizer the yields of beets in tons per acre and in sugar while fairly high were not significantly affected by the irrigation treatments. These investigators, however, concluded that with fertilizer, the yields were influenced by the variations in soil-moisture contents.

Doneen (1942) concluded from his studies on the relation of soil moisture to growth and nutrition of sugar beets on a fertile loam soil that growth is independent of soil moisture as long as readily available moisture is present in the soil. He found that varying the amount of readily available moisture did not affect the nitrate-nitrogen of the soil and that the nitrogen content of the roots and leaves and the total nitrogen removed by the crop is not influenced by the variations of soil moisture used in his experiments.

Doneen, Porter & MacGillivray (1939) found with watermelons that wide variation in soil moisture (in one case the soil was at the permanent wilting percentage for $1\frac{1}{2}$ months during the latter part of the growing season) did not affect yields, growth of plants, and melons.

Résumé

In considering the responses of plants to variations in soil-moisture contents the permanent wilting percentage of the soil under test should be indicated. The amount of energy required to remove unit mass or volume of water from a soil will indicate whether water is available to plants if the value of the free energy or potential of the soil moisture at the time at which plants wilt is known. In this case, the reporting of moisture conditions on an energy instead of a moisture scale will serve the same purpose.

Physical measurements show that the energy required to remove water from the soil changes materially as the moisture content decreases, but it does not follow that the availability of the water to plants also decreases. By far the greatest drop in energy in the soil-moisture plant system occurs at the surface of the leaf cell walls which surround the substomatal surface as Gradmann (1928) and Edlefsen (1942) have pointed out. The latter, from Thut's (1939) data, has calculated the drop in free energy between the leaf tissues and the outside air of -9.231×10^8 ergs per gm. The total free energy of the water surrounding the roots at the permanent wilting percentage may be taken to be about -0.16×10^8 ergs per gm. At 40 per cent relative humidity, that of the air is -9.4×10^8 ergs per gm. or an overall drop of -9.24×10^8 ergs per gm. from the soil to the air. The increase in energy required when the soil moisture is reduced from the field capacity to the permanent wilting percentage is unimportant when the system as a whole is considered.

The reason that plants wilt may be explained by the position of the permanent wilting percentage on the energy soil moisture curve in the region where a slight decrease in moisture content results in a great increase in resistance to removal of the water. Failure of the water supply to the plant, of course, may be due to the slowness of movement of water into the mass of soil dried by the roots.

Another cause of water deficiency at soil-moisture contents near the permanent wilting percentage may be the failure of roots to elongate rapidly enough into regions where there is still water above the permanent wilting percentage.

Whether water is readily available to plants or not in the final analysis must be decided by empirical experiments. While the results of growing plants in containers may indicate trends, they should not be taken as being conclusive unless confirmed by field trials.

One difficulty with plants in the field is the sparse root development of some plants. Sometimes, either due to soil conditions or inherent characteristics of the plants, roots will not thoroughly permeate the soil. Consequently, neither soil sampling nor measurements of soil properties which are related to soil-moisture contents, made with physical instruments inserted into the soil, will give reliable records of the actual moisture content of the soil in contact with the absorbing portion of the roots. Thus, erroneous conclusions may be drawn.

Much of the material not reviewed does not contain sufficient data to permit an analysis because they were based on techniques which obviously are faulty. For instance, those in which it is purported to maintain a predetermined moisture in the soil in which plants are growing were not given consideration.

The results of investigations on the relation of plant growth to soil moisture show that the plants grow well throughout a wide range of soil moisture, but some investigators question whether they do so with equal facility throughout the entire range from field capacity to permanent wilting percentage. The permanent wilting percentage is the most important soil-moisture constant. The accuracy of its determination is highly important.

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SOIL CHEMISTRY IN RELATION TO INORGANIC NUTRITION OF PLANTS

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INTRODUCTION

Plant physiologists, through studies with water culture techniques, have firmly established certain important facts regarding the mineral nutrition of plants that have a direct bearing on concepts of the soil as a medium for plant growth. Some of the most interesting and far reaching facts are: first, the solid phase of soils in itself is unnecessary for optimal growth of plants; second, organic matter per se is wholly unnecessary; third, although the dominant mass of the soil consists of alumino silicate minerals, neither aluminum nor silicon have been found to be essential for plant growth; fourth, microorganisms as such and their activities are unnecessary; and lastly, the same elements are essential for the growth of most, if not all, species even though different ones may vary greatly in their ability to absorb essential or nonessential elements from soils or other culture media.

Soil scientists have assumed the responsibility of securing and classifying knowledge of soils. In doing so they have developed at least four phases of specialization, i.e., soil morphology, soil physics, soil microbiology, and soil chemistry. The accumulated knowledge of soil phenomena is highly complex in detail and involves both living and inert systems. No reasonably integrated interpretation of soil systems as related to growth of plants neglects the dynamics of interactions between soil, plant, and soil microorganisms as modifying factors continually contributing to adjustments within the soil medium.

Soil chemistry touches every phase of soil science in all of its complexity, but in seeking a general chemical approach to problems in plant nutrition a simple yet effective point of reference is provided in the knowledge that 15 essential elements can be made into a suitable medium for plant growth. However, the kinds of nutritional problems coming within the perspective of the soil chemist are very broad and must embrace nonessential as well as essential elements. Moreover, in order to understand the soil as a medium for plant growth, he finds it necessary to look into the structures of soil minerals, for the types of dominant soil minerals in themselves influence the power of soils to supply essential mineral elements. Also, since the object of plant production is largely to provide food for animals, he frequently carries his thinking and his activities into the realm of animal nutrition.

CHEMICAL ELEMENTS ABSORBED BY PLANTS

Chemical elements of soils are absorbed by plants whether they are physiologically required in plant functions or not. In all probability, if soil-grown plants were analyzed with sufficiently sensitive chemical methods all elements founds in the earth's crust would appear as plant constituents. The reason for this is simply that a definite mechanism for absorption exists and that soluble inorganic materials are sufficiently similar to participate in absorption processes. It may be noted that plutonium, a recently synthesized chemical element, was found by Overstreet & Jacobson (1) to be absorbed and translocated by plants.

Despite some overlapping it is convenient to place the elements of interest to soil chemists in categories as follows: (a) the familiar elements essential for plant growth; (b) elements forming the dominant mass of the soil—silicon, aluminum, titanium, iron and oxygen; (c) elements essential for the nutrition of animals but which are not required by plants—sodium and chlorine which are required by the animal in quantity, and iodine and cobalt which are required only in minute amounts; (d) elements exercising a marked influence on the physical state of the soil mass by reason of differentially effecting the dispersive or flocculating tendencies of soil colloids—hydrogen, sodium, potassium, calcium, and magnesium; (e) elements which have been found in soils usually in such small amounts that they exercise a very minor rôle with respect to the physical properties of soils, but which have been found to be toxic or deleterious to the health of plants or animals—boron, fluorine, arsenic, selenium, nickel, manganese, molybdenum.

A direct nutritional rôle for inorganic rock constituents is seen in essential minerals derived by soils from their parent rock, 11 of the 15 known essential elements being chiefly from this source. Depending on relative quantities of specific elements required for normal plant nutrition, the elements essential for plant growth are frequently referred to as macronutrient or micronutritent elements. In keeping with concepts of a general chemical approach to problems of plant nutrition, the rock-derived nutrient ions may be divided into four groups, each deserving special consideration, namely, the macronutrient cations—calcium, magnesium, and potassium; macronutrient anions—sulfates and phosphates; micronutrient cations—iron, manganese, zinc, and copper; and the micronutrient anions—borate and molybdate. Rapidly expanding interest and increasing research resulting from the general recognition of the importance of micronutrient element nutrition in plants and animals has been one of the outstanding scientific developments of the last 20 years (2).

SOIL NITROGEN AND ATMOSPHERIC SOURCES OF OTHER ESSENTIAL ELEMENTS

All of the essential elements may be regarded as ultimately originating from rocks except hydrogen, oxygen, carbon, and nitrogen. These are contributed more or less directly by the atmosphere and are used by plants in the forms of water, molecular oxygen, carbon dioxide, and ammonium or ni-

trate nitrogen. Of this group all, except perhaps carbon dioxide, must be present in the soil and even though soils frequently contain high amounts of carbonates, only negligible amounts are assimilated by plant roots (3). The need for water in soils is obvious, and is discussed in another chapter in this volume. Oxygen as an essential component of the root environment has a constant reserve in the atmosphere. Its rate of supply is limited solely by physical factors determining the rate of transfer of gases of the atmosphere with those of the soil moisture.

Of the atmospheric elements, nitrogen is unique inasmuch as it cannot be used by plants until it is transformed from molecular nitrogen to nitrate or ammonia by agencies such as atmospheric electricity or nitrogen-fixing organisms. Reduced or oxidized forms of nitrogen are known as "fixed" nitrogen. Nitrate nitrogen is highly soluble in water and is not known to be adsorbed by any of the solid phase materials of soils. On the other hand, reduced or amino nitrogen accumulated in the organic fraction of soils is highly insoluble and as such constitutes an important reserve of soil nitrogen. The most stable form of soil organic matter, or humus, is a material of indefinite composition, but in all soils it tends to assume a somewhat constant proportion of carbon to nitrogen. It is relatively resistant to attack by soil microorganisms as compared to most organic plant constituents. The tendency to approach constant proportions of carbon and nitrogen arises from the fact that plant lignins persist in soils and by condensation with decomposition products of microbial protoplasm form the nitrogen-containing humus (4). Nitrogen transformations in soils are, therefore, exceedingly complex and manifestly associated with living plants, their dead and decaying remains, and the activities of soil microorganisms. However, the forms of nitrogen absorbed by plants are the simple inorganic ammonium or nitrate ions. From the standpoint of mineral nutrition, ordinary practices of fallowing increase the power of soils to produce crops largely because of inorganic nitrogen derived from soil organic matter. During the growing season soils ordinarily show a marked decline in inorganic nitrogen because it is rapidly absorbed by plants (5, 6). When the inorganic nitrogen reaches low levels of a few parts per million, significant daily fluctuations have been observed (6) which were caused by differential rates of microbial release of organic reserve nitrogen and absorption by plants. Undisturbed soils tend to approach an equilibrium state with respect to soil organic matter and fixed soil nitrogen (7) as a result of secular competition between all agencies contributing to fixation and those removing soil nitrogen. Many controversial attitudes regarding the maintenance of soil fertility result from incomplete understanding of the significance of soil organic matter as a reserve of nitrogenous material built up only after years of accumulating plant residues (8).

Since this paper deals with soil chemistry as related to inorganic plant nutrition, we choose to include soil nitrogen with the other inorganic plant nutrients, realizing that the major quantities of absorbable nitrogen appearing in soils will be either as a cation in the form of ammonium ion or as nitrate, an anion.

THE SOIL SOLUTION

Closely associated with the establishment of mineral requirements of plants by water culture studies, much knowledge concerning the soil as a medium for plant growth was obtained by studies of the soil solution and of water extracts of soils (5). There is appealing simplicity in the idea that only the liquid phase of soils need be considered as the source of supply of essential plant nutrients, but one of the principal results of early investigations was that soil solution frequently did not contain enough nitrogen or phosphate salts to constitute an adequate supply of these elements unless they were continually resupplied to the soil moisture. Indeed, it is through examination of soil solutions and water extracts of soils that knowledge of the rôle of soil microorganisms in replenishing the inorganic nitrogen supply has been elucidated.

Whether or not there is such a thing as a true soil solution will long be debated. In the light of information regarding adsorbed soil ions, it is manifest that ionic concentrations at the surface of soils differ in kind and amount from those more distantly located in the body of the soil liquid. Any soil capable of supporting plant growth must have in it a source of supply of nutrient anions as the means of supplying sulfur, phosphorus, nitrogen, boron and molybdenum. At least sulfates, phosphates, and nitrates are always found in soil solutions extracted from fertile soils (5). An equivalent number of dissolved cations must, therefore, always be present in the same liquid. Wet soils must have water molecules surrounding surface adsorbed ions as well as the dissolved salts. Obviously any technique for separating the liquid phase of soils from the solid phase will be unable to remove surface adsorbed ions. The question of what actually constitutes the true soil solution can, therefore, only be answered in terms of description of a heterogeneous liquid phase whose boundary concentrations at the solid-liquid interface have not yet been experimentally determined.

Under the circumstances it is the opinion of the writers that the term "soil solution" should apply to the liquid contained in soils which can be separated and whose chemical characteristics can be accurately measured. For this reason we choose always to refer to "displaceable soil moisture and its dissolved salts" as the soil solution. A satisfactory method for displacing soil liquid was suggested by Parker (9) and further developed by Burd & Martin (5, 10) in their classical studies of the chemical nature of the soil solution. More recently Richards (11) has devised another method for separating soil moisture, which permits extraction of the soil solution at soil moisture contents below those that would give rise to permanent wilting of plants. Reitemeier & Richards (12) have compared the chemical compositions of solutions extracted by the pressure membrane apparatus with Burd's (10) displacement method over a wide range of soil moisture contents for a variety of different textural grades of soils from sands to heavy clays. They have concluded that the same solution is extracted by either technique. Since two totally different methods used to separate soil moisture give rise to solutions of the same composition, credence is given to the idea that

despite theoretical reservations, a reasonably discrete and homogenous soil solution does occur, at least at salt concentrations of the order of 10 m.eq. per liter or greater.

Perhaps the most critical work on the chemical nature of soil solutions has been that of Reitemeier (13) who separated the liquid phase from soils of varying proportions of water with pressure membrane equipment. The moisture contents of each soil investigated ranged from approximately 500 per cent water to somewhat less than the normal field capacity.

There are numerous important points concerning soil solution concentrations illustrated in the curves given in Reitemeier's paper, which deserve detailed study by anyone working in soil chemistry, particularly those interested in the soil as a medium for plant growth. For our purposes, however, it may be emphasized that the soluble salts were found to be qualitatively and quantitatively different for every moisture content assumed by a given soil. Moreover, the solubility patterns exhibited were characteristically those of particular soils. For example, with some soils, soluble components such as calcium or magnesium increased as water was added to the soil, whereas other soluble components remained constant or decreased. With another soil the pattern exhibited by similar components was quite reversed. Intermediate trends were shown by still other soils wherein addition of water first caused more of a particular ion to enter the solution and later, as further water was added, caused them to leave the soil solution. Even highly soluble components, such as nitrates and chlorides, exhibited an apparently anomalous behavior inasmuch as at lower soil moisture contents they increased in concentration much more rapidly than can be simply accounted for by considering all soil water to be equally capable of dissolving salts. Nitrates and chlorides may thus be said to exhibit "negative adsorption" (14), a phenomenon many times reported but not studied over a wide range of soil moistures. In Reitemeier's (13) work this phenomenon was clearly shown to be significant and was characteristically expressed by all of the soils investigated. As a result of his observations we now know that the concentrations of nitrates and chlorides of soil solutions at field moisture may be from 20 to 30 per cent higher than calculated from extracts using large amounts of water. Furthermore, his curves indicate that nitrates and chlorides increased in concentration at a much more rapid rate in soil solutions falling below field capacity toward the wilting point than in soil solutions which approached the field capacity from higher soil moisture contents.

SALINITY OF SOIL SOLUTIONS IN RELATION TO PLANT GROWTH

From the point of view of plant growth on saline soils excessive salts dissolved in the soil solution assume immediate importance. It is here that the significance of salt concentrations *per se* can best be understood, since it is obvious that there must be some physiological limit to the plant's ability to absorb water from solutions of high osmotic pressure. Although physical factors such as capillarity ordinarily dominate the total water stress in soils (15), the osmotic pressure of the soil solution proper is additive.

Recently, Wadleigh & Gauch (16) have experimentally shown the existence of precise relationships between the rate of plant growth and the total moisture stress in soils. Their experiments were conducted with cotton plants under both saline and nonsaline soil conditions. Regardless of physiological age and subsequent inherent differences in growth rate, all growing leaves on the cotton plants were found to be directly influenced by the intensity of soil moisture stress which included the osmotic pressure of the soil solution. In spite of the difficulty of quantitatively evaluating factors involved in the growth of plants in so complex a medium as the soil system, their data of cumulative leaf length as a function of soil moisture stress were highly regular and consistent, enabling them mathematically to relate the two variables in parabolic equations. Growth of leaves of different physiological age were found to differ in their relationships to moisture stress only in the constants of the equation; but the derivatives of all equations from which the total moisture stress could be calculated at zero growth rate, were found to be between 14 and 15 atm. Broyer's (17) theoretically derived relations between the relative volumes of cells and hydrodynamic and osmotic forces has been observed by Wadleigh & Gauch to find a parallel in their experimentally established curves for elongation of leaves of cotton plants at different soil moisture tensions. It is highly interesting that a theoretical treatment of physical chemical factors which should be expected to determine the turgor and subsequently the volume of cells has been so closely expressed in plant growth experiments. The work of Wadleigh & Gauch therefore, strongly suggests that growth, or enlargement of plant tissues, if a direct function of turgescence, must be partially conditioned by the forces in the soil system tending to withhold water from the plant. Thus, the colligative properties of soil solutions of different concentrations, although not directly concerned in the supply of inorganic plant nutrients, represent an important phase of soil chemistry having to do with their utilization.

Correlations between high salt concentrations of the soil solution and unsatisfactory growth of agricultural crops have been shown by Magistad & Reitemeier (18). They have found that when the osmotic pressure of the soil solution, measured at the soil moisture percentage giving rise to permanent wilting, exceeded 4 atm. crops were abnormally low in yield even when ample water was supplied. Since the moisture content of soils at the permanent wilting percentage is on the average about half that of the field holding capacity (15), most of the plant growth would have to take place between 2 and 3 atm. On the other hand, they found that if soil solutions were less than 4 atm. osmotic pressure at the permanent wilting percentage, irrigated crops grew well, particularly if nitrates constituted a substantial portion of the salt.

In 1938 the U. S. Department of Agriculture established a Regional Salinity Laboratory at Riverside, California to study problems of crop production in semiarid regions (19). Control of salinity in agricultural lands is a large scale chemical process, which depends for its continued success upon complete chemical knowledge of the movement of different kinds and

amounts of salts in soils. Where ideal chemical or climatic conditions cannot be realized for all crops, physiological studies of salt tolerance of different plant species as influenced by climate and different kinds of salts are helpful in choosing appropriate crops for soils unavoidably burdened with higher salt levels. For example, whereas much evidence points to osmotic pressure as a factor in plant nutrition, salts such as chlorides have been shown by Hayward et al. (20, 21) to be somewhat more injurious than sulfates. Peach trees, for example, which made some growth at 3.6 atm. in a culture dominantly composed of sulfate salts, died at 3.4 atm. if chlorides were substituted for sulfates. Magistad et al. (22) have also shown that climates with high temperatures and high light intensities are more apt to induce salt injury in a given plant species when grown on the same nutrient substrate

Soil Solutions of Fertile Soils—Comparison with Culture Solutions

Generally, osmotic pressures of successful nutrient culture solutions are low—of the order of 1 atm. or less. Similarly, soil solution concentrations of fertile soils are also found to be of the same order of magnitude. However as indicated earlier the two principal departures of soil solutions from the composition of an adequate nutrient solution are found in the concentrations of soil nitrates and phosphates. Because soil nitrates are entirely contained in the soil solution (13) and are rapidly absorbed by plants it can be stated that the ability of soils to support plants depends in part upon the rate at which nitrates removed from the soil solution can be replaced by biological oxidation of solid phase organic matter (5). Thus, the ability of the soil to supply inorganic nitrogen to the soil solution at an adequate rate to meet the physiological demands of growing plants assumes more importance than the total amount of nitrogen in the solid organic phase, or the amount of nitrate nitrogen found in solution at any given time, as may be revealed by chemical analysis.

Concentrations of phosphate in soil solutions are unique in that they are always low—of the order of 1 p.p.m. of solution. In view of the demonstration by Arnon & Hoagland (23) that excellent and equally high yields of crop plants can be obtained from fertile soil, solution cultures, or sand cultures when grown in the same climatic environment, it is not difficult to conceive of the soil solution with its dissolved salts as being analogous to a culture solution. This analogy rests on the assumption that the low concentrations of materials such as phosphate in the soil solution can be maintained to satisfy the requirements of plants. Tidmore (24) in experiments with flowing cultures and large volumes of culture solutions has shown that corn, sorghum, and tomatoes can in fact secure adequate amounts of phosphate from solutions containing 0.5 p.p.m. of soluble phosphate. Solution concentrations of 1 p.p.m. resulted in luxury consumption by plants without increased growth. Should one view the soil solution as the sole source of phosphate for soil grown plants, it would be necessary to account for renewal of soluble phosphates many times during the growing season. For example, in

greenhouse experiments with 2 kg. of soil in pots we have grown 20 gm. of dry plant material containing 1 per cent phosphate in a six-week period. The total phosphorus absorbed was, therefore, 200,000 μg. Since the soils were maintained with about 400 cc. moisture containing 1 p.p.m. phosphate, the soil solution at any one time contained about 400 μg. of soluble phosphate Consequently, the forty-day old plants had to absorb, on the average, 5000 μg. phosphate each day. In order to account for the total phosphate extracted from the soil solution, it is necessary to postulate complete renewal of the soil solution phosphate on the average of ten times each day. Obviously the newly germinated plants would have a much lower demand for phosphorus than larger plants later on. The figures cited for rates of renewal are, therefore, conservative. Other calculations applied to field conditions also indicate that soil solution phosphate must be renewed several times daily in order to account for actual amounts of phosphate removed by crops.

Although macronutrient ions such as phosphate, nitrate, and frequently potassium, may attain very low levels in the soil solution, concentrations of the micronutrients are undoubtedly much smaller. Critical investigations of soil solution concentrations of the micronutrient elements are very meager principally because there are no convenient chemical methods for assaying the small amounts involved, but it will be shown later that studies of micronutrients adsorbed by the cation (25) and anion (26) absorption complexes in soils show considerable promise of bringing our understanding of these difficult elements to a level with our knowledge of macronutrients. New techniques, using high specific activity radio isotopes (27, 28), have increased the sensitivity of detection of small amounts of manganese (25), zinc (27), and molybdenum (28) which make it possible to conduct quantitative studies of the absorption of these elements from soils or culture solutions at the characteristically low concentrations of physiological significance in plant nutrition. The most comprehensive and general study so far undertaken in the direction of determining soil factors influencing the concentration of the micronutrients in equilibrated suspensions of soil materials is that of Epstein (25) who has shown that the rates of absorption by plants of micronutrient cations—iron, manganese, zinc, and copper—were closely related to the proportion of exchange spots occupied by them. He also showed that the concentration of solution cations was similarly determined by the amounts adsorbed on the solid phase. Concentrations of active ions in both the solid and solution phases are mutually interdependent as is discussed in detail in the subsequent section on *Cation exchange.*

ORIGIN OF SOLUBLE MATTER FROM THE SOLID PHASE

Inorganic salts.—The solid phase of a dry soil contains materials which dissolve to a varying degree in the soil moisture as water is added. As already indicated in the earlier part of the discussion of the soil solution very complex interrelations exist between different ionic species coming into solution. For purposes of simplification, four types of solid salts are sufficiently distinct in their properties to deserve separate consideration in accordance with their

solubilities. The four pertinent classifications, with some of the more obvious examples of each, are listed:

(a) completely soluble salts, such as nitrates or chlorides;

(b) sparingly soluble salts, $CaSO_4 \cdot 2H_2O$, $Ca_3(PO_4)_2$, $CaHPO_4$, $CaCO_3$, $MgCO_3$, $FePO_4$, $AlPO_4$;

(c) insoluble salts, $Ca_3(PO_4)_2$, $BaSO_4$,

(d) colloidal salts, commonly referred to along with colloidal acids as the "exchange complex."

Obviously the choice of relative terms ascribing solubility is only a matter of custom. It will be noted that $Ca_3(PO_4)_2$ has been mentioned in two different categories, and may be thought of as belonging to either one or the other, depending upon its state of subdivision, the "soil pH," and so on. In the final analysis, all solid materials are soluble and, with sufficient time, continued leaching with pure water should result in their complete solution.

The clay complex.—The familiar exchange complex or adsorption complex of the soil chemist has been listed above as a colloidal salt. Although the latter term is not generally used, it should be pointed out that the adsorption complex does have properties analogous with those of salts; for example, the ability to conduct electrical current and to give rise to a base and a colloidal acid upon electrolysis. Generally the colloidal half bears a negative charge which can be entirely compensated by positively charged hydrogen ions or other cations. When the negative charges of the colloid are satisfied by hydrogen ions it is frequently referred to as a colloidal acid; or, since clays provide most of the reactive ions of soils and constitute the inorganic colloidal fractions of soils, the terms clay acid or hydrogen clay (30) are used synonymously.

Clay acids can be titrated directly with strong bases such as NaOH or $Ca(OH)_2$ giving rise to sodium clays or calcium clays and, during the progress of such a titration, exhibit the properties of a weak acid (30) as reflected by typical buffer curves. Treating acid soils with lime ("liming the soil") effects such a chemical reaction and it may be written:

$$Clay]^-_-{}^{H+}_{H+} + Ca^{++} + 2OH^- \rightleftharpoons Clay]^-_-Ca^{++} + 2H_2O$$
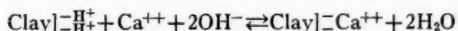
or simply, $H_2X + Ca^{++} + 2OH^- \rightleftharpoons CaX + 2H_2O$ where X represents the adsorption complex which is of colloidal nature. Assignment of an appropriate charge to X is of paramount importance in determining the proper equilibrium constants associated with the above and other reactions and will be amplified later in the section on *Cation exchange.*

Soil clays also participate in anion exchange reactions, in a manner somewhat similar to cation exchange reactions, but differing in that strongly adsorbed hydroxyl ions may be exchanged for other anions. Anion exchange reactions are not entered into by ions of the soil solution nearly as readily as cation exchange reactions, but are of great importance in determining the fate of the strongly adsorbable phosphates and molybdates. Chlorides and nitrates rarely participate in such exchanges, and sulfates do so but slightly. In order to obtain anion exchange it is necessary to have free hydrogen ions present in the system which, by reason of the energy of formation of water,

assist in removal of the tightly held hydroxyl ions. An example of an anion exchange reaction involving phosphate is given below.

$$X]^+OH^- + H^+ + H_2PO_4^- \rightleftharpoons X]^+H_2PO_4^- + H_2O$$
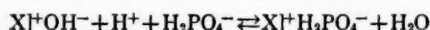
From the above equations it is seen that the composition of the soil solution at any time must reflect the integrated results of the action of water on a great many salts of different inherent solubilities. The salts in turn may participate in cation and anion exchange reactions with the relatively insoluble solid phase soil material. For a detailed discussion of inferences of such interreactions, the reader is referred to Reitemeier's paper (13).

CHEMICAL DENUDATION

Since soil moisture always contains dissolved salts, any water carried away from the soil and into drainage systems represents a net loss which may be called chemical denudation. Although the process is not obvious, it is continually in progress in all land areas receiving rainfall. Moreover, the amounts of soluble materials removed annually are relatively large when one thinks in terms of fertilizers that might be added for improved crop production.

Geochemists (31), taking into account the volume of flow and the salt concentrations of the principal rivers of the world's great drainage basins, have arrived at the figures for chemical denudation given in Table I.

TABLE I*

CHEMICAL DENUDATION OF LAND AREAS AS DETERMINED FROM
SALINITY AND VOLUME OF FLOW OF MAJOR RIVERS

Continental area	Area drained, millions of sq. miles	Tons of salt removed per sq. miles	Millions of tons of salt removed per year	Approximate yearly removal-pounds of salt per acre
North America...........	6	79	474	250
South America...........	4	50	200	150
Europe..................	3	100	300	300
Asia....................	7	84	588	250
Africa..................	8	44	352	140

* Compare ref. (31, p. 115).

It will be seen in this Table that the average annual loss of soluble material ranges from 140 to 300 pounds per acre for the entire drainage basin. Another way of visualizing the magnitude of gross chemical denudation is found in other calculations (31) which show that one foot depth of the entire

land surface of England and Wales would have to disappear every 13,000 years in order to account for soluble salts carried to the ocean by streams.

As large as these effects of weathering appear to be on the scale of geological time, all soils of the earth's surface, as far as is known, retain enough of the essential elements to support some kind of plant life. Complete consideration of the different factors concerned in soil evolution (32) must allow for geological events effecting the cyclic renewal of parent material exposed to surface weathering such as wind and water transport, volcanism, and the raising of land masses from beneath oceans, etc.

Accelerated denudation.—Figures showing the integrated result of chemical denudation for an entire drainage basin, though useful for purposes of orientation, do not take into account variations within different parts of the drainage area where the intensity of weathering may vary enormously. Regions or localities of high rainfall, for example, provide water in such quantity that nearly all simple salts are removed and essential elements such as calcium, magnesium, and perhaps potassium, held in adsorbed forms on the exchange complex are exchanged for hydrogen ions. The hydrogen ions originate from the water, but are provided in quantity through carbon dioxide accumulated from the atmosphere by plants and released in the soil through root respiration and the activities of soil microorganisms. The amounts of carbon dioxide provided in this way are large indeed, since it is not uncommon for a heavy plant cover to fix 1,000 pounds in a single month. Because of the relatively high proportion of adsorbed hydrogen ions appearing in the exchange complex of soils of wet climates, they are known as "acid soils." Since calcium, magnesium, and perhaps potassium, may be reduced to levels below those required for the optimal growth of plants, acid soils are also frequently found to be infertile when subjected to cultivation. In the process of chemical denudation, climatic temperatures and types of plant cover also exercise a marked influence on differential rates of removal of silicon with respect to iron and aluminum. Soils in cold humid climates under coniferous forests are characterized by high silica contents, whereas humid tropical regions give rise to soils with very little silica, the dominant mass of the soil being composed of the hydrous oxides of iron and aluminum, and kaolinitic alumino silicates, all of which are capable of fixing large amounts of phosphate in the anion exchange complex. This latter fact is mentioned because the mineralogical nature of the exchange complex is highly important in considering the fertility of soils from an agricultural point of view.

Restricted and intermediate denudation.—On the other hand, very little salt can be removed from the soil mass if rainfall is too limited to permit a substantial portion of rains to enter streams, or if drainage is impeded. Under some circumstances, as found in semiarid regions, a zone of accumulated salts appears in a lower soil horizon at the average depth reached by soil moisture. These and similar situations frequently are found to give rise to salt accumulations too great for unrestricted plant growth. Soils developed in semiarid climates are, therefore, often saline or alkaline. Regions having

intermediate rainfall and drainage may be found to strike a balance such that the products of chemical weathering are removed in the drainage waters just fast enough to prevent salt accumulation without depleting the soil of essential minerals. Such soils are neutral or slightly acid.

From the point of view of soil fertility, therefore, an understanding of soil chemistry as related to salt movements permits reasonable interpretations of the suitability of soils to support plant growth in situations which might appear to be quite anomalous to the casual observer. For example, when climatic conditions are highly favorable for continuous support of plant life as in tropical rain forests, dense plant covers may be established even though conditions favoring chemical denudation are extreme. When these soils are brought into cultivation, organic matter in the absence of proper safeguards, is rapidly oxidized. The remaining inorganic soil material is infertile by comparison with an acceptable standard for fertile soils in temperate regions. Viewing the dense foliage of the tropics, one might well consider the sparse vegetation of semiarid regions as evidence of infertile soils. To the soil chemist, however, the accumulated mineral wealth of unleached semiarid soils is most impressive and he justifiably entertains expectations of their high potential productivity if irrigated and properly managed. Thus, in tropical soils plant nutrients are likely to limit plant growth, whereas in semiarid areas it is the supply of water which frequently limits crop production.

Farmers, as individuals familiar with the necessity of conducting agricultural enterprises with limited capital, seek regions where past climatological environments have given rise to soils of intermediate chemical characteristics, where good crops can be grown without the necessity of irrigation or adding supplementary fertilizers. Such soils do exist but they are very rare. Virgin grasslands of the prairies would perhaps serve as one of the better examples. However, after being placed under cultivation, their history, like nearly all newly cultivated lands, has been one of slowly declining fertility. Thus sooner or later, the desire to improve or to maintain crop yields presents problems of soil chemistry in relation to plant nutrition.

EXCHANGE CAPACITIES OF SOILS AND THEIR SIGNIFICANCE IN PLANT NUTRITION

Cation exchange capacity.—The maximum number of adsorbed ions held by a given weight of soil material is known as the cation exchange capacity of the soil, usually expressed as milliequivalents of adsorbed ions per 100 gm. of dry soil material. Another, more recent practice has been to express exchange capacities as equivalents per million (e.p.m.) (13, 19). The latter expression differs from the former by a factor of 100. Thus, the cation exchange capacity of a soil may be, for example, either 23.5 m.eq. per 100 gm. or 0.235 e.p.m.

One might look upon a material such as muscovite mica as representing a layer lattice alumino silicate of ultimate high cation exchange capacity since of the natural layer lattice minerals, it contains a great number of iso-

morphically substituted aluminum ions within tetrahedra. From the formula $H_2KAL_3Si_3O_{12}$ and considerations of the structural position of potassium at the cleavage planes of the layer lattice, all of the potassium should become exchangeable if made accessible to the liquid phase. If sufficiently subdivided, the maximal figure for exchangeable potassium in muscovite would, therefore, be 2.42 e.p.m. or 242 m.eq. per 100 gm. Practically speaking, such subdivision is difficult to attain although Kelley & Jenny (33) were able to show that muscovite ground in a ball mill released 52.5 m.eq. of potassium when leached with neutral ammonium salt solutions. Ammonium ions adsorbed in the exchange process amounted to 48.3 m.eq. per 100 gm.

Natural clays such as montmorillonite commonly show exchange capacities ranging from 70 to 120 m.eq. per 100 gm., but those of kaolinitic clays may be very much lower—5 to 20 m.eq. It is manifest that natural soils containing different amounts and different kinds of clay minerals should vary greatly in their cation exchange capacities. Sandy soils, for example, may have exchange capacities as low as 1 or 2 m.eq. by reason of the absence of clay material, whereas peat soils, by reason of the nature of their exchange complex may have capacities of as much as 80 or 90 m.eq. per 100 gm.

Many methods for determining the cation exchange capacity of soils have been proposed (34). The methods are empirical, and usually consist of leaching the soil material with neutral salts. Neutral ammonium salts such as 1.0 N NH_4Cl or 1.0 N NH_4-acetate are the most common reagents and are preferred because the exchange capacity can be determined directly by distilling off the ammonia from an alkaline suspension of the ammonium saturated soil. Since ammonium or acetate ions are but rarely normal constituents of soils, the normal replaceable cations and salts may be determined by direct analysis of the ammonium acetate leachate.

Anion exchange capacity.—The mechanism of anion exchange is to be discussed later in this paper in connection with the structure of clay minerals. It will be seen that the process in itself is not strictly comparable to cation exchange processes inasmuch as a substitution of an anion in the surface of the crystal lattice is required for every hydroxyl ion replaced. The substituted ion must be of a size that fits into the lattice without steric hindrance. Anions meeting these requirements are phosphates, arsenates, fluoride, and hydroxyl ions.

Because of the importance of phosphates as essential nutrient ions, anion exchange reactions involving phosphate become of greater importance than others. In special cases arsenates of spray materials may be fixed by soils to the advantage of crops which might otherwise suffer from arsenic toxicity.

Following the suggestion of Burd & Murphy (35) and Murphy (36) that the saturation capacity of soils for phosphates should be considered in diagnosing phosphate supplying power of soils, Piper (37) tentatively proposed a method for determining anion exchange capacities of soils by leaching them with normal solutions of ammonium phosphate adjusted to pH 4. Adsorbed phosphates were then determined by replacing with sodium hydroxide and analyzing the solution for displaced phosphate. The same procedure was

used in earlier studies (38) designed to obtain the maximum possible replacement of phosphate ions for the hydroxyl ions of minerals of known composition. The method is, therefore, analogous to that used for the determination of cation exchange capacities, in that a high concentration of a replacing salt accompanied by leaching is used in order to effect complete exchange. Rubins & Dean (39) have modified the procedure by pretreating the soil with acid sodium acetate solutions to remove calcium salts, followed by an anion replacing reagent of normal sodium phosphate adjusted to pH 5.7. The hydrogen ion concentration was thus made to correspond more nearly to the pH values of phosphate-deficient acid soils. They have used their method with good success in diagnosing the phosphate supplying power of acid soils (40).

Significance of the degree of saturation of the exchange complex.—If adsorbed ions constitute an immediate reservoir of soil-borne nutrients such as the nutrient cations, and the nutrient anions, phosphate, molybdate, and borate—there obviously exists some limit below which these nutrients cannot be supplied in adequate amounts to satisfy the physiological requirements of plants. Nutritional significance of adsorbed ions expressed in terms of the total cation exchange capacity was suggested by Hoagland & Martin (41), who found that potassium deficiencies were more frequently observed in plants if the exchangeable potassium fell below 1 per cent of the total base exchange capacity. Jenny & Ayres (42) worked out more precise relationships for the adsorption of potassium by plants from clay suspensions over a wide range of adsorbed potassium levels, and were thus able to explain the potassium nutrition of plants in accordance with the degree of saturation of the clay complex with respect to the adsorbed ion in question.

Herein lies an important problem for the student of soil-plant interrelations that has not yet been solved by chemical studies of soils, nor by solution cultures with plants. Adequate mineral nutrition of soil-grown plants depends upon factors other than the total amounts of mobile ions present in soils and consideration must be given to the relative proportions of specific cations adsorbed on soil surfaces and to the kinds of dominant clay minerals (43).

Special considerations of adsorbed calcium.—Calcium is the principal adsorbed cation found in agricultural soils and many converging lines of evidence indicate that the calcium levels of natural soils are frequently of consequence in the calcium nutrition of plants. Thorne (44) studied relations between calcium and sodium and potassium with controlled cultures wherein the cations were supplied to the plants in adsorbed form from bentonite (montmorillonitic) substrates. Calcium uptake by plants was found to be progressively decreased with diminishing degrees of calcium saturation, being little different whether the complementary ion was potassium or sodium. Pronounced injury to plants was found when sodium occupied 50 per cent of the exchange complex which was ascribed to a special injury due to sodium. However, all plants growing in cultures having a degree of calcium saturation of less than 35 per cent showed a sharp drop in calcium

uptake, which Thorne suggested might have been the result of a breakdown of the calcium regime. Corroborative evidence of the necessity of high adsorbed calcium levels for adequate calcium nutrition of plants was obtained by Arnon & Grossenbacher (45). With synthetic resins containing adsorbed cations they observed that furnishing nutrient cations in adsorbed form in amounts equal to those of an adequate sand or solution culture, resulted in crop failure accompanied by acute calcium deficiency symptoms. A degree of calcium saturation of over 30 per cent was required for plant growth in Amberlite resins.

Rosette disease of lettuce on several soils of serpentine origin and high in exchangeable magnesium was clearly shown by Vlamis & Jenny (46) to be the result of calcium deficiency. A similar view is held by Walker (47, 48) who, by straightforward exchange processes, reconstituted several kaolinitic soils of serpentine rock origin to provide calcium levels ranging from 5 to 80 per cent of the exchange capacity with the principal complementary ion being magnesium. Increasingly severe calcium deficiency symptoms appeared on tomatoes and lettuce for calcium levels diminishing below 25 per cent. However, Streptanthus, an adaptive endemic species of serpentine soils, was able to acquire adequate calcium from levels as low as 10 per cent of the exchange capacity. Mehlich & Reed (49) have shown that adequate calcium nutrition of peanuts, as evidenced by the percentage of nuts filled, was provided by kaolinite having 22 per cent calcium on the exchange complex, but the same degree of saturation was quite inadequate if organic colloids were substituted. Other direct physical-chemical evidence of a higher calcium activity of kaolinite-adsorbed calcium than that of montmorillonite-adsorbed calcium at equivalent degrees of calcium saturation has been shown by Marshall (50) from calcium activity measurements using semipermeable clay membrane electrodes.

A similar approach to problems of supply of the micronutrient cations has been taken by Epstein (25). Using radioactive isotopes of high specific activity, he was able to obtain accurate assays of the amounts of adsorbed cations of iron, manganese, and zinc taken up by plants in 24-hour absorption periods. He concluded that the principal factor determining their uptake by plants was the degree of saturation of the clay. Varying proportions of complementary ions of the macronutrient cations such as calcium and hydrogen were of much less importance. With manganese above 0.10 per cent saturation, amounts absorbed by plants were not specifically related to the mol fraction (51) nor to the quantities formulated by Krishnamoorthy et al. (52). However, below approximately 0.10 per cent saturation for iron, manganese, and copper, and below 0.20 per cent for zinc, the clay-adsorbed micronutrient cations were made available to plants in amounts much more nearly proportional to the activities of the clay-adsorbed micronutrients.

Soil phosphates of the anion adsorption adsorption complex also appear to be amenable to the same kinds of considerations. For example, Burd & Murphy (35) found that increasing growth of tomatoes was obtained

up to 30 per cent of the phosphate absorption capacity of kaolinitic substrates. Also, Dean & Rubins (40) in analyzing over 100 samples of acid soils for exchangeable phosphorus concluded that the amount of exchangeable phosphorus in itself was not a reliable index of phosphate fertility, but rather the degree of saturation. It was pointed out (40) that soil phosphate extracted by Peech's (53) modification of the familiar Truog method (54) for testing for plant available soil phosphates was directly related to the exchangeable phosphorus and its degree of saturation, but inversely related to anion exchange capacity. This suggests to the reviewers that the success of the method (53) in evaluating the phosphate supplying power of acid soils depends upon securing figures reflecting the degree of saturation of soil colloidal materials.

The remarks regarding the importance of the degree of saturation in assessment of nutrient element nutrition of soils should not imply too great a degree of simplification of the total problem of soil plant interrelations since in many soils calcium, phosphorus, and the micronutrient cations are all capable of forming important amounts of sparingly soluble salts. Leeper (55), for example, calls attention to the many ramifications of soil manganese chemistry, further complicated by microbiological oxidations and reductions. The general problems of soil phosphate chemistry have been recently discussed by Burd (56) who points out that the important phosphate chemistry of neutral and alkaline soils containing large amounts of calcium largely involves insoluble calcium phosphate salts and is entirely different from that of acid soils. Also, many intermediate soil conditions exist which simultaneously involve both types of soil phosphate systems. It should also be borne in mind that we have omitted from this discussion the very important organic soil phosphates, shown by Wrenshall & Dyer (57) to consist largely of phytin, nucleic acids, and other organic phosphorus compounds not clearly identified.

It is to be hoped that exchange equations once firmly established in theory, and shown to be applicable with consistency at all levels of saturation, will become a powerful tool for approaching such problems. However, at the present time, direct experimental work with the complete dynamic plant-soil system must be depended upon to provide facts for making available workable hypotheses of the chemical levels of the nutrient elements in soils of significance to plant nutrition.

Special considerations of soil potassium.—It might appear incongruous that potassium, normally obtained by plants from the exchange complex of soils, should so often require special terms to describe apparent departures from normal chemical or colloid chemical reactions. Thus, besides exchangeable and soluble soil potassium, we recognize (58) "nonexchangeable potassium," "nonexchangeable potassium available to plants," "nonexchangeable potassium fixed in moist soils," and "nonexchangeable potassium fixed by drying." More recently Attoe (59) has observed nonexchangeable potassium becoming exchangeable upon drying the soil. These peculiar phenomena do not apply to calcium, magnesium, or sodium ions, but are characteristic

of rubidium (58) and ammonium (60) ions. Usually there is a stoichiometric reduction of cation exchange capacity in an amount equal to the potassium fixed (58).

From the considerable evidence now at hand, it would appear to the writers that the various phenomena associated with the fixation and release of potassium can best be explained on the assumption that clay minerals of micaceous habit are characterized by an increased tendency to hydrate as the negative charge on the layer lattice is decreased below that of the true micas and biotities. Intralattice ionic exchange takes place rapidly in completely expanded lattice of minerals such as montmorillonite, but much more slowly in the nonexpanding hydrous micas because of the crystalline nature of the intralattice water. In the nonexpanding hydrated materials, potassium slowly migrates to the outer edges of the crystals where it can participate in cation exchange, and be absorbed by plants, and would thus represent "nonexchangeable potassium available to plants." Substituted calcium, magnesium, or sodium ions occupying intralattice exchange spots do not provide the necessary steric properties to support the micaceous habit of the layer lattice crystal, and these ions are readily exchangeable. However, upon addition of excess ammonium, potassium, rubidium, cesium, and perhaps hydronium ions to solutions in contact with the soil, they promptly exchange with adsorbed calcium, magnesium, or sodium ions. The substituted monovalent ions, being of a proper size and charge, effectively permit crystallization of the warped and slightly expanded members of the layer lattice into a more compact micaceous habit. This latter event would be recognized as the rapid "fixation of potassium in nonexchangeable form." It has been shown by Reitemeier et al. (61) that "nonexchangeable potassium available to plants," can be released by laboratory methods such as continued moist contact, alternate freezing and thawing, digestion with 1.0 N HNO_3 and by electrodialysis. Particularly good correlations were obtained between the amount of nonexchangeable potassium absorbed by pot cultures of ladino clover grown for 740 days with the amounts released by electrodialysis for 30 days. With some of the soils tested, more than half of the potassium absorbed by ladino clover originated from nonexchangeable forms.

Soil pH.—The hydrogen ion status of different chemical types of soils is universally recognized as having implications as to their fertility. There is perhaps no simpler and more direct way of obtaining an initial clue to the chemical characteristics of a soil than by determining its pH. In practice, soil pH measurements are nowadays almost universally made with some type of glass electrode instrument. Such measurements are made on water suspension of soils or even by packing moist soil around the electrode. Since the soil is a multiphase system, the term "soil pH" does not have intrinsic meaning in itself but only reflects the fact that an instrument capable of determining the pH of solutions also records an electromotive force (EMF) when immersed in soil suspensions. Usually the EMF obtained from a soil suspension does not greatly differ from that obtained from a clear filtrate of the suspension. If the "suspension pH" always showed the same electro-

metric measurement as the solution with which it is in equilibrium, one could speak of the "soil pH" without reservations. However, this is not always the case. Under special conditions, particularly in laboratory preparations of hydrogen clays, the "suspension pH" may be several pH units different from that of the intermicellular liquid. This may readily be shown by making separate pH measurements of the two phases after filtration or centrifugation (30).

It is not difficult, for example, to visualize a two phase system, consisting of only a hydrogen clay suspended in water. Although the pH of the water itself is 7, an electrode assembly inserted into the suspension of hydrogen clay definitely measures an acid reaction. The mechanism by which the EMF is created is still a matter receiving the attention of soil chemists. The problem is by no means solved, since it may be complicated by membrane potentials not subject to direct measurement, and by liquid-suspension junction potentials which may be of large magnitude as compared to liquid-liquid junction potentials encountered in true solutions (62). A complete explanation of the actual mechanism involved will have to account for the fact that soils giving an acid "suspension pH" always contain adsorbed hydrogen ions, and that greater proportions of adsorbed hydrogen ions on a given soil are reflected in a more acid "soil pH."

In view of the lack of an adequate theory of the reactions involved in the measurement of "soil pH," it is well to exercise caution in making a precise interpretation of the results of such measurements in terms of physiological responses of plants to hydrogen ion concentration as ascertained by nutrient solutions studies. Experience has shown that plants may grow well in soils having soil pH values from as low as pH 4.5 to as high as 8.5. The older view that most plants have distinct optimal pH values for growth has not been supported by solution culture studies (63). Our more recent knowledge is that plants tolerate a wide range of pH values and can make good growth provided mineral nutrients are available in absorbable form (64).

It is much more likely therefore that observed "soil pH" effects on plant growth are in reality due to secondary changes in chemical compounds from which mineral nutrients are derived. Notably at alkaline reactions insoluble compounds of nutrient elements are formed. Most obvious are the less soluble carbonates and phosphates of calcium and magnesium. Also at alkaline reactions, the carbonates, oxides, and hydroxides of the micronutrient metals—iron, copper, manganese, and zinc—are least soluble.

In acid soils other indirect effects are induced tending to alter the availability of mineral nutrients. Moreover, the mere fact that soils are acid means that hydrogen ions have taken the place of nutrient cations on the clay colloids. Calcium, in particular, is required by plants at relatively high levels, a point which has already been emphasized in this review. Another aspect contributing to infertility of some acid soils has to do with excessive solubility of compounds of manganese and perhaps of aluminum, which may enter soluble or adsorbed forms in amounts great enough to be toxic.

In view of the lack of any physiological basis for sharply defined hydrogen

ion preference of plants (63) and the complicated nature of different kinds of soils, it is the opinion of the reviewers that classification of plant species into acid-loving, lime-loving, etc. is not generally warranted. Although practical correlations of this sort may be established in a certain geographical region where chemical soil types are sufficiently alike, they may not necessarily be valid in other geographical regions or for other types of soils.

Transfer of ions from soil to plant; contact exchange.—Plant scientists have long been interested in the influence of the plant on the heterogeneous soil environment, and have logically considered carbonic acid originating from plants as effectively accounting for continued solubilization of solid phase materials. Thus it appeared that plants absorbed cations from the soil solution by replacing them from the exchange complex with hydrogen ions originating from the carbonic acid of respiration and from such other biological sources of hydrogen ion in soils as nitrogen fixation and transformations of organic sulfur, phosphorus and nitrogen compounds by microorganisms. Although this was a seemingly satisfactory hypothesis to explain most of the facts regarding plant-induced changes in soil solutions, there remained the possibility that it did not completely account for detailed relationships existing between roots and solid-phase materials of soils.

Jenny & Overstreet (65) have pointed out that inasmuch as both plant roots and soils have colloidal properties, the laws governing the chemistry of interreactions between colloids must also be applicable to the soil-plant system. Thus they have postulated the contact exchange of ions between roots and the solid phase as a necessary consequence of viewing soil and plant systems as materials having characteristic surface reactions.

Although many practical difficulties stand in the way of obtaining experimental proof for this theory, data have been accumulated tending to indicate that direct exchanges within intermingling electrical double layers of the root and soil systems may take place without the necessity of postulating an intermediate sojourn of ions in the soil solution. Jenny (66) has recently reviewed the status of contact phenomena in the mineral nutrition of soil-grown plants. Wadleigh & Bower (67) have shown that the mineral absorption by plants from culture solutions is greatly different from the absorption from solutions of the same composition, but where the plant roots were also in contact with solid phase exchange materials. Experiments with artificial culture solutions are of great value in providing the basic information as to mineral requirements of plants, but a more detailed understanding of the significance of the soil as a medium for plant growth must depend on growth media containing solid-phase materials whose colloid chemical properties are subject to experimental control.

Use of chemical analysis of soils in determining fertility.—From the foregoing discussions it is seen that soils in general are characterized by widely different chemical properties. Moreover, all ranges of fertility levels may occur on any one chemical type of soil. Crop plants, as well as other species, differ remarkably in the extent of their root systems, in growth rates, and in climatic requirements. Rates of supply of nitrogen involve not only soil

factors but also the activity of microorganisms. These, as well as other variables such as soil-water relations and salinity problems, have invariably ruled against the validity of universally applicable chemical methods capable of accurately assessing the fertility levels of soils. The principal use of chemical analyses, therefore, is to be found in establishing the chemical nature of soils with respect to exchange capacities, the kinds and amounts of sparingly soluble or soluble salts, and the mineralogical types of absorbents.

In determining fertility levels of particular nutrient elements by "quick" chemical tests almost any method of extracting nutrient elements from soils is capable of distinguishing between soils of very high and very low fertility, but unfortunately this kind of information is of little help in farming operations where close calculation of expected yield per unit of added fertilizer is required.

The many different types of chemical examinations of soils which have been proposed, or which are currently used, are too numerous for inclusion in this review. It may be stated, however, that greater degrees of success with chemical soil tests have been found in areas in which factors of soil type, climate, and crop are sufficiently alike to permit the establishment of useful correlations between chemical tests and crop growth.

One of the more recent trends in soil fertility estimations has been toward plant analysis. Since the mineral content of the growing plant itself is the result of integration of the factors of its soil and climatic environment and of its own genetic powers of absorption, chemical tests of plant tissue should be indicative of the plant's own degree of success in extracting an adequate mineral supply from the soil in which it is growing.

For details of this and other chemical, biological, and chemobiological methods of testing soil fertility levels, reference is made to recently published reviews (68).

THE CLAY MINERALS

As with all complex subjects, soil chemistry has progressed periodically with the advent of new discoveries which led to better understanding of the fundamental nature of mechanisms or processes. In 1926, when soil clays were thought to be amorphous gels of indefinite composition, it was suggested by Russell (6) that the use of x-rays might disclose as much about the structures of soils as had been learned about the structure of crystals by the Braggs, father and son (69, 70). The Braggs, through elucidation of principles governing formation of crystals, had been able to solve the long-standing puzzle of different empirical formulae of seemingly similar crystalline alumino silicates. They showed that most alumino silicate minerals were built around frameworks of silica tetrahedra, and aluminum tetrahedra or octahedra formed by surrounding the relatively small silicon and aluminum cations by oxygen or hydroxyl ions. Thus, from a structural point of view at least, alumino silicate crystals were found to be far simpler than might be supposed from considerations of complex empirical formulae alone. Oxygen

ions like other anions of alumino silicates, were found to be large compared to cations; for example, compare the ionic radii of the principal anions O^{-2}, 1.32 Å; F^-, 1.33 Å; OH^-, 1.4 Å with those of cations of more than one charge; S^{+6}, .34 Å; P^{+5}, .34 Å; Si^{+4}, 39 Å; Al^{+3}, .57 Å; Fe^{+3}, .67 Å; Mg^{+2}, .78 Å; Fe^{+2} .83 Å (71). Relative sizes of oxygen ions and silicon ions may be directly compared in their characteristic structural arrangements in Figure Ih. Four large oxygen ions placed compactly together in tetrahedral form provide space at the center to accommodate the small tetravalent silicon ion (figs. Ia, Ib). Since the silicon of complex alumino silicate minerals was always found to be located within such groupings of oxygen ions it became known as the silicon tetrahedron, and silicon is said to have a coordination number of 4. Similar tetrahedral structures of the same size characteristics of SiO_4^{-4} are the familiar PO_4^{-3}, SO_4^{-2}, and MnO_4^- radicals. Aluminum ions are somewhat larger than silicon ions but can be constrained to the same sized tetrahedron. Structures containing aluminum are more stable, however, if surrounded by six oxygen or hydroxyl ions, the resulting configuration being in the form of an octahedron (figs. Ic, Id). Octahedral aluminum, surrounded by six anions, has a coordination number of 6, but if aluminum is substituted in a tetrahedron, it has a coordination number of 4. Thus, different ionic species having the same sign charge and not differing by more than 15 per cent in size, may occupy similar positions within crystal lattices. It is not necessary that the magnitude of the charge be identical. The larger interstice of the octahedron is capable of accommodating a large number of cations of similar size particularly in the loose packed arrangement of Figure Id. In alumino silicates, magnesium ions (.78 Å) very commonly occur in octahedra, but not calcium ions, which are too large (1.08 Å). Many of the heavy metals including iron may be freely substituted in octahedral positions. The phenomenon of substitution of different ionic species within spatial positions capable of accommodating them by reason of their size is known as "isomorphic substitution." The ease of incorporating many different elements into single crystals does much to explain why all soils, ultimately originating as they do from molten magma, are provided with all of the rock-derived elements required for plant growth including the micronutrients (29).

Pauling's classic solutions of the structures of micas (72) as typical layer lattice alumino silicates, provided the final link required for the present understanding of the significance of the crystalline nature of soil colloidal materials. The following year the discovery that soil clay materials were in fact crystalline and not amorphous was made independently by Hendricks & Fry (73) and Kelley, Dore & Brown (74). Many studies of structures of the clay minerals, and others of the implications of crystal chemistry to soil interactions, have been made since that time. For details, the reader is referred to excellent reviews on the subject by Hendricks (75), Kelley (34, 76), and Grim (77).

Since the clay materials of soils are largely crystalline in nature, it becomes fairly easy to identify them specifically by means of x-ray diffraction patterns. In its simplest aspect, identification is accomplished simply by

separating the clay materials, from larger fractions of the soil securing their x-ray diffraction patterns, and comparing the diffraction lines so obtained with those of known minerals. It is a remarkable fact that the clay fractions of soils which are responsible for most of the immediate chemical reactions of importance to plant growth, are relatively few in number. Moreover, they are rarely the same minerals, merely subdivided, of the original parent minerals from which they were derived and thus must be secondary products of synthesis. Specifically, soil clays may be classified as to their dominant mineralogical composition as belonging to the montmorillonitic, kaolinitic, or hydrous mica groups. In a rough way, the intensity of weathering seems to be a much more important feature in determining the crystallographic nature of clay materials developed in soils than their parent material. Alkaline and neutral soils are often dominated by montmorillonite, neutral to moderately acid soils by hydrous micas and more intensively acid soils by kaolinitic alumino silicates. Generalizations regarding the formation sequence of soil clay minerals have been proposed by Jackson et al. (78) who point out that clays are polycomponent, their total composition tending to be in the form of a distribution curve with the two dominant minerals indicating the position of the clay in the weathering sequence. It is their contention that hydrous mica breaks down into montmorillonite which subsequently loses silica to form kaolinitic alumino silicates—the last stage of the weathering cycle. In the interim, silica or hydrous oxides of iron and aluminum also accumulate, sometimes to make up almost the entire soil mass. Recent studies by Barshad (79) may have considerable bearing on revealing further relationships between the formation of soil minerals through cation exchange reactions. He has shown that vermiculite, a natural hydrated mica-like mineral, can be converted in the laboratory to a mineral closely approximating biotite when leached with potassium salts. Other conversions accomplished were biotite and hydrobiotite to vermiculite by leaching with magnesium salts. Of particular importance was his finding that the conversions could be accomplished with macroscopic particles. Thus for layer lattice alumino silicates as exemplified by hydrous micas, vermiculite, and perhaps biotite as well, essential cations are available from particles of much larger sizes than the official dimensions of clay.

Structures of the layer lattice alumino silicates and the seat of cation exchange.—A highly significant aspect of soil minerals may be derived from the structure of a macroscopic crystal of mica, a familiar material because of its many industrial uses as an insulator and as a flexible, transparent, heat resistant sheet. Mica is a typical layer lattice alumino silicate. It cleaves readily and may be subdivided by splitting into thinner and thinner sheets, the only limitation to ultimate thinness being the skill of the operator and the precision of his tools. Theoretically, the smallest possible sheet would be 10Å or 10^{-7} cm. in thickness, which is the size of the neutral packages or sheets of which the mica crystal is composed. The sheets have high tensile strength in all lateral directions, since along the extended dimensions of the crystal it is held together by coulomb charges, or ionic bonds.

Mica and many of the other layer lattice alumino silicates have very nearly the same structural configuration, although their empirical formulae may appear to have little in common; for example, pyrophylite $H Al Si_2O_6$, muscovite (potassium mica), $H_2KAl_3Si_3O_{12}$, paragonite $H_2Na Al_3 Si_3O_{12}$, and talc $H_2Mg_3Si_2O_6$ (33). All four of these minerals are formed by stacking electrically neutral sheets in layers, as indicated by their ease of cleavage. Individual sheets are held together by weak interraction of the electrons and nuclei of the ions of one alumino silicate sheet with those of the other. The binding force between layers is the close range electronic van der Waal's attraction, best exemplified in the condensation of rare monatomic gases to liquids at sufficiently low temperatures.

As with all alumino silicates, the skeletal outline of the crystal is provided by oxygen and hydroxyl ions arranged in tetrahedral and octahedral groupings with the large anions being shared by more than one tetrahedron, or simultaneously with a tetrahedron and several octahedra, as may be deduced from Figures Ia to Ie and its accompanying explanation.

Formulae for pyrophyblite, talc, and muscovite can be written showing the number of ions in each atomic plane which result in an electrically neutral layer lattice. As represented below, there are seven atomic planes (separation being indicated by a colon). The third and fifth planes contain both oxygen and hydroxyl ions. Differences between pyrophyllite, talc, and muscovite are seen to arise through isomorphic substitution.

(a) pyrophyllite—$O_6:Si_4:O_4(OH)_2:Al_4:O_4(OH)_2:Si_4:O_6$

(b) talc—$\qquad O_6:Si_4:O_4(OH)_2:Mg_6:O_4(OH)_2:Si_4:O_6$

(c) muscovite— $KO_6:Si_3Al:O_4(OH)_2:Al_4:O_4(OH)_2:Si_3Al:O_6K$

The successive additions of the seven atomic planes of pyrophyllite may be followed directly through Figures h, i, j, l, m, and n in FIG. I.

In the pyrophyllite structure there are empty octahedra beside those occupied by aluminum ions. In talc they are filled by magnesium ions. Thus, lattice layers of talc differ from those of pyrophyllite in that six magnesium ions are substituted for four aluminums in the fourth atomic plane (note the unoccupied central position within the hexagonally arranged small white aluminum ions, fig. Ij). Muscovite layers differ from those of pyrophyllite in that aluminum has been isomorphically substituted for silicon in one of every fourth tetrahedron of the second and sixth atomic planes (figs. Ih, Im). The extra negative charge left over by substituting trivalent aluminum for tetravalent silicon is balanced by a potassium ion which occupies the space within the center of the hexagonally linked ring of oxygen ions in the first and seventh planes (figs. Ie, Ih, In). This space is empty in pyrophyllite, but occupied by potassium in mica.

In the layer lattice, plate-like clays of soils, it is highly unlikely that complete and uniform substitutions of the kind indicated above are ever accomplished. Rather, they are characterized by considerable heterogeneity in isomorphic replacements. Moreover, there may be interleaving of layers of different intrastructural properties. Of principal importance, however, is the fact that isomorphic substitution of aluminum for silicon in tetrahedra,

gives rise to a negatively charged surface, which if accessible to the liquid phase may be satisfied by any cation, and, therefore, may be visualized as one of the important seats of the cation exchange capacity of soils. Consequently, if the number of substitutions in a given clay mineral is high, the cation exchange capacity is also generally found to be high as in vermiculite (79).

An interesting property of some clay materials is found in their ability to imbibe water between cleavage planes and to swell. Montmorillonite is the most characteristic soil mineral exhibiting this behavior. It is of such interest to students of soil chemistry that the early work of Hoffman, Endell & Wilm (80) deserves mention in this review. Through x-ray diffraction patterns they were able to follow the expansion of the layer lattice as water was adsorbed. In montmorillonite, it was found that the forces tending to hold water on the intralayer surfaces were great enough to force the individual layers apart. As increasing amounts of water were adsorbed, the layers expanded until the thicknesses of the water layers were greater than the montmorillonite layers themselves. Upon drying, interplanar water was lost until the original layer lattice was restored. Gieseking (81) and Ensminger & Gieseking (82), in studies of the nature of adsorption of organic materials by soil clays, have used montmorillonite crystals essentially as calipers of molecular dimension. Since the negatively charged intralayer surfaces of montmorillonite reacted with amino groups as well as with simple cations, they were able to adsorb organic compounds containing amino groups, including proteins, upon montmorillonite. Upon drying the material, the montmorillonite layers were held apart by reason of the presence of the adsorbed organic compounds. X-ray measurements of lattice spacing then provided them with a direct measurement of the thickness of the adsorbed organic material.

Kaolinite and similar end product layer lattice alumino silicates in relation to anion exchange phenomena.—Kaolinite has been mentioned earlier as an end product of the weathering sequence (78). It is also noted that it is associated with the hydrous oxides of iron and aluminum. Thus nearly all minerals subjected to long and intensive weathering are characterized by the presence of surface hydroxyl ions. Of importance to soil chemists considering problems of plant nutrition is the fact that intensively weathered soils nearly always present problems, sometimes exceedingly acute, in phosphate nutrition. It is becoming increasingly clear that the dominant soil minerals formed under the circumstances represent an enormous reservoir of hydroxyl ions which are exchangeable for phosphates and other nutrient anions such as molybdates.

The structure of kaolinite (83) and associated minerals such as halloysite (84) shows that each layer lattice is characterized by an entire sheet of hydroxyl ions as illustrated by the black balls of the model of kaolinite shown in Figure Ik. The sequence of atomic planes composing a neutral kaolinite layer may be directly compared with those of pyrophyllite in the formulae below:

(a) pyrophyllite—$O_6:Si_4:O_4(OH)_2:Al_4:O_4(OH)_2:Si_4:O_6$
(b) kaolinite— $O_6:Si_4:O_4(OH)_2:Al_4:(OH)_6$

Whereas pyrophyllite has seven successive atomic layers and is completely symmetrical, kaolinite has but five and is unsymmetrical. The five atomic planes of kaolinite corresponding to the second formula above are shown

a   b
tetrahedra

c   d
octahedra

e - linked
tetrahedra

f - subst. PO₄
tetrahedron

g - phosphated
surface

h - 1st and 2nd
atm. planes

i - 3rd atm.
plane added

j - 4th atm.
plane added

k - KAOLINITE
5th plane added

l - 5th plane of
pyrophyllite

m - 6th plane of
pyrophyllite

n - PYROPHYLLITE
7th plane added

o - KAOLINITE
side view
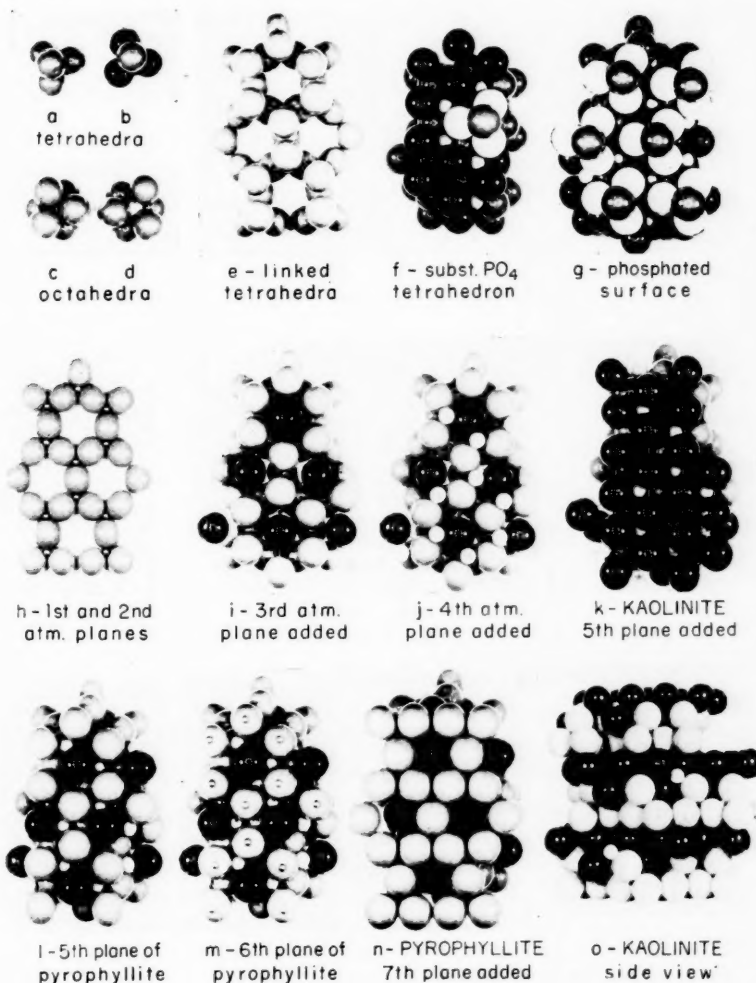
Fig. I. Structural features of the layer lattice alumino silicates.

being built up in sequence in h, i, j, and k of Figure I. This sequence may be directly compared with h, i, j, l, m, and n, resulting in the formation of the symmetrical pyrophyllite lattice. A possible method of effecting anion exchange is shown in Figures If and Ig. Figure If shows a phosphate tetrahedron substituted for three hydroxyl ions in the fifth atomic plane of kaolinite where it fits without steric hindrance, and where each oxygen of the substituted phosphate tetrahedron obtains half of an effective coulomb charge from each of the two aluminum ions in touch with it in the atomic plane below. It was shown by Stout (38) that when lattice hydroxyl ions of kaolinite and halloysite are exposed, they become accessible for exchange with phosphate ions. Black (85) has indicated that the slow reversion of soluble phosphates in the presence of kaolinite can be accounted for by slow intralattice penetration of phosphates exchanging for hydroxyl ions of the alumino silicate, although in a later paper (86) he indicates that the silica may be replaced instead.

Many workers have investigated phosphate adsorption by soils, and have regarded one of the responsible mechanisms of fixation as one involving anion exchange. Anion exchange in soils should not be considered as strictly analogous to cation exchange, since the exchangeable hydroxyl ions are a part of the crystal lattice of the soil colloid. When the hydroxyl ions are replaced by other anions the new ion assumes a position on the crystal lattice more suggestive of an isomorphic substitution. Of the very many investigations of soil phosphate reactions, the most critical work directed toward the significance of the contribution of anion exchange reactions is that of Dean & Rubins (87). They have shown that soils have an anion exchange capacity in the legitimate sense of the word as evidenced by the fact that alternate saturation with arsenates and phosphates could be effected. Adsorbed phosphates could also be replaced by fluoride and hydroxide. Citrates removed adsorbed phosphates but were not in themselves adsorbed. Although the fact that soil colloidal materials exhibit anion exchange properties seems firmly established, the rôle of the anion exchange complex in the mineral nutrition of plants needs to be further elucidated. At present the concept of adsorbed phosphates in soils appears to have a productive future in explaining the phosphate nutrition of plants growing in acid soils having large amounts of hydrous oxides of iron and aluminum, and kaolinitic silicates.

CATION EXCHANGE EQUILIBRIA

Until recently the phenomenon of cation exchange has been regarded with little interest outside of agriculture, where its importance was early appreciated (88) as a mechanism for retaining plant nutrients, particularly potassium and ammonium nitrogen, against loss to drainage waters. In recent years, however, ion exchange processes have received wide application in chemical industries and many chemists interested in other processes than those of soil chemistry have turned their attention to this interesting matter of interreactions between adsorbed and dissolved ions. Since chemical phenomena taking place in soils can best be approached through under-

standing the factors governing solid phase—solution phase equilibria, the problem has been treated through equations representing various equilibrium conditions which permit exact definition and measurement.

The process of cation exchange, or "base exchange" as it is frequently referred to in the earlier soils literature, concerns the fixation or adsorption on the surfaces of the soil particles of inorganic ions of the solution phase and also their release to the soil solution. As mentioned earlier the nutrient cations of some soils may be almost entirely present in the adsorbed state on the surfaces of the soil particles. Since the solid particles bear a permanent negative charge, the adsorbed cations can be released only by exchange for other cations. Normally the plant effects the release of the nutrients by means of hydrogen ions originating from plants which subsequently replace the adsorbed ions and set them free from the particles.

The process whereby adsorbed cations are released from solid particles by exchange for other cations is called the "cation exchange process." In soils it must, of course, take place between the colloidal clay particles and the soil solution. Cation exchange is stoichiometric and can be represented by means of equations as follows:

$$\boxed{}\ Ca^{++}+2Na^{+}\rightarrow\ \boxed{}\ \begin{matrix}Na^{+}\\[2pt]Na^{+}\end{matrix}\ +Ca^{++}$$
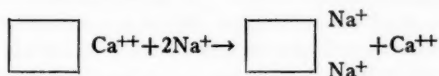
where the rectangle represents the clay particle.

Very often the clay particle is regarded as a monovalent anion and the exchange is represented as follows:

$$CaX_2+2Na^{+}\rightarrow 2NaX+Ca^{++}$$
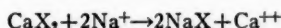
the clay particle being represented by the symbol X.

As normally encountered in soils, base exchange processes are reversible and equilibrium is attained rapidly. In the following sections we shall be concerned with the formulation of the equilibrium conditions for this very important process in soils. This formulation has a very practical value as well as a theoretical interest. As will be pointed out later, the nutrition of a plant in soil depends not only on the composition of the soil solution but also on the relative proportion of the nutrient ions in the adsorbent phase of the soil. This being the case, it is often found desirable to alter the proportions of adsorbed nutrients in a soil so as to promote better growth of crops. From the standpoint of practical agriculture this can be done only by means of soil amendments. Upon additions of salts to soils the ion exchange process always becomes involved. For example, in the case of alkali soils it is found that the proportion of sodium ions in the absorbent phase is undesirably high when it is above 15 per cent of the total adsorbed cations. This condition is remedied by the addition of calcium salts to the soil, whereupon the following ion exchange process takes place:

$$2NaX+Ca^{++}\rightarrow CaX_2+2Na^{+}.$$
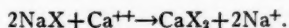
The sodium ion thus freed from the particles can be leached from the soil with irrigation water.

Since the discovery of the base exchange process in soils by Way (88) in 1850, there have been many attempts by soil chemists to represent the equilibrium conditions of the process by means of mathematical equations. The equations that have been developed fall roughly into three classes, according to the line of reasoning used in their formulation; namely, (a) purely empirical equations, (b) equations developed from kinetic considerations, and (c) equations based on the law of mass action.

The empirical cation exchange equations.—If, for example, increasing amounts of potassium chloride are added to a suspension of a soil that contains only calcium ions in the adsorbed state, the following reaction will proceed:

$$\boxed{}\; Ca^{++}+2K^{+}\rightarrow \boxed{}\begin{matrix} K^{+} \\ \\ K^{+}\end{matrix} +Ca^{++}.$$
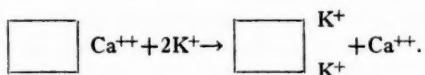
With each addition of potassium chloride the amount of potassium adsorbed at equilibrium can be represented by a continuous curve.

If the experiment is carried to high additions of potassium, we find that the curve resembles a hyperbola—it approaches a maximum corresponding to the exchange capacity of the soil present. On the other hand, if only small additions of potassium chloride are made, it will not be possible to determine whether the curve is a hyperbola or a parabola.

A number of empirical equations for ion adsorption have been proposed by Wiegner & Jenny (89), Rothmund & Kornfeld (90), Vageler & Woltersdorf (91) and others. These equations are all either parabolic or hyperbolic and perhaps do not warrant extensive description here. However, empirical equations of this type often are of value in the representation of experimental data. For this reason, two such empirical relationships which are often used will be mentioned.

The first of these is a parabola of the form, $y=kx^{n}$, where x and y are variables and k and n are constants. This equation is commonly referred to as the Freundlich Isotherm because it is similar in form to the equation developed by Freundlich ($x/m=kp^{1/n}$) for the adsorption of a gas on a solid, such as carbon dioxide on charcoal. The equation can be written in the alternate form

$$\log y=\log k+n \log x.$$

Thus if $x=$ m.eq. of ion added and $y=$ m.eq. of ion adsorbed, then the log of the amount of ion adsorbed is a linear function of the log of the amount added over the experimental range where Freundlich's equation is applicable.

The second empirical equation is hyperbolic and has the form

$$y=abx/1+ax$$

where x and y are variables and a and b are constants. This equation is often referred to as the Langmuir Isotherm beacuse of its similarity to the equation derived by Langmuir for the adsorption of a gas on a solid i.e. $x/m = abp/\mathrm{I} + ap$.

The last equation can be put in the more useful form,

$$abx/y = ax + \mathrm{I},$$

so that if $x = $ m.eq. of the ion added and $y = $ m.eq. of the ion adsorbed, then a linear relationship is obtained between the quotient, m.eq. added per m.eq. adsorbed, and the m.eq. added where this type of equation is valid. It is to be noted that the constant b of the equation corresponds to the adsorption maximum or the cation exchange capacity of the adsorbent used.

Although a variety of experimental facts can be described by means of the empirical ion adsorption equations, they are not theoretically satisfying since they have not been derived from assumptions concerning the nature of the adsorbing surface and the mechanism of the exchange process. In this respect kinetic ion exchange equations are more acceptable.

Exchange equations derived from kinetic considerations.—A kinetic ion exchange relationship that has received wide attention is that derived by Jenny (92). Jenny's original treatment dealt with exchanges between ions of the same valence; that is, with base exchange reactions of the type

$$\boxed{}\,b^+ + w^+ \rightarrow \boxed{}\,w^+ + b^+.$$
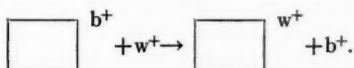
Jenny assumed the surfaces of the soil particles to be planar in character and having a definite number of attraction spots per unit area. He also assumed that each adsorbed ion oscillates about its attraction spot within a space called the "oscillation cell," the average volume of which is a characteristic volume, V. Thus, an adsorbed potassium ion is characterized by an oscillation volume V_{K^+}, an adsorbed sodium ion by a volume V_{Na^+}, etc.

Jenny assumed further that exchange can take place only when a free ion from the solution phase comes between an adsorbed ion and its attraction spot. From probability considerations he calculated the rates of adsorption of each of the two monovalent ions under consideration in terms of the total number of exchange spots, the amounts of each ion, their oscillation volumes, and the total volume of the system. By equating the exchange rates of the two ions at equilibrium he obtained an expression of the form,

$$W = [(S+N) \pm \sqrt{(S+N)^2 - 4SN(\mathrm{I} - V_w/V_b)}]/2(\mathrm{I} - V_w/V_b).$$

$W = $ number of w ions adsorbed (usually expressed in per cent of S), $N = $ number of w ions added to b-clay, $S = $ cation exchange capacity of clay, $V_w = $ oscillation volume of adsorbed w ions, $V_b = $ oscillation volume of adsorbed b ions.

Jenny subjected the foregoing equation to experimental test, employing a number of ion pairs of equal valence and a variety of soil colloids. He found

that the equation represented the equilibrium conditions quite satisfactorily, except for the case of ion pairs involving hydrogen ion.

In recent years a second kinetic equation has been developed by Boyd, Schubert, & Adamson (93) in researches with synthetic ion-exchange resins. Boyd and co-workers based their equation directly on Langmuir's (94) theory for the adsorption of two gases on a solid. They considered first the most simple case of the simultaneous competitive adsorption of two singly charged cations A^+ and B^+. Then by formal analogy with Langmuir's theory for adsorption from a binary gaseous mixture they found that the equation for the adsorption of one of the ions, A^+, from a dilute electrolyte solution is

$$(x/m)_{A^+} = k b_1 C_{A^+}/1 + b_1 C_{A^+} + b_2 C_{B^+}$$

where $(x/m)_{A^+}$ = amount of A^+ ion adsorbed per unit weight of adsorbent, C_{A^+} and C_{B^+} = respective equilibrium concentrations of ions A^+ and B^+ in solution, k, b_1, b_2 = constants.

To a good approximation, the quantity unity in the above equation can be neglected relative to the quantity $(b_1 C_{A^+} + b_2 C_{B^+})$. With this approximation, the equation can be expressed in the following form:

$$C_{A^+}/C_{B^+}/(x/m)_{A^+} = b_2/b_1 k + 1/k \cdot C_{A^+}/C_{B^+}.$$

Thus it can be seen that the quantity $C_{A^+}/C_{B^+}/(x/m)_{A^+}$ is a linear function of the quantity C_{A^+}/C_{B^+} where this treatment is applicable. Boyd *et al.* demonstrated the suitability of the foregoing formulations for the adsorption of a variety of ions on synthetic resins.

In general, it can be said that the equations based on kinetic considerations have proved quite satisfactory in describing the exchange process between ions of equal valency on a mineralogically pure adsorbent; a frequent exception being ion pairs involving the hydrogen ion. Thus far the kinetic theories have not been satisfactorily extended to include the case of ion pairs of unequal valence. In this respect certain approaches based on the law of mass action have been far more fruitful.

Exchange equations derived from the law of mass action.—The mass law formulations are based on the treatment of the ion-adsorption process as a chemical reaction as exemplified in the following equations: $K(ad) + Na^+ \rightleftharpoons Na(ad) + K^+$, $Ca(ad) + 2Na^+ \rightleftharpoons 2Na(ad) + Ca^{++}$, $La(ad) + 3Na^+ \rightleftharpoons 3Na(ad) + La^{+++}$ where the suffix (ad) denotes the adsorbed condition. The corresponding equilibrium constants in terms of the "activities" of the reactants and products are:

$$a_{Na(ad)}/a_{K(ad)} \cdot a_{K^+}/a_{Na^+} = k_1$$

$$(a_{Na(ad)})^2/a_{Ca(ad)} \cdot a_{Ca^{++}}/(a_{Na^+})^2 = k_2$$

$$(a_{Na(ad)})^3/a_{La(ad)} \cdot a_{La^{+++}}/(a_{Na^+})^3 = k_3$$

The problem of calculation of equilibrium constants for the exchange process thus lies in the evaluation of the activities of the ions in the solution phase and in the adsorbent phase.

Where the solution phase of the soil or soil suspension is very dilute the activities of the constituent ions (a_{Na^+}, a_{K^+}, $a_{Ca^{++}}$, etc.) can be assumed equal to their respective molalities. Where the soil solution is more concentrated the appropriate activity ratios usually can be evaluated by means of the principle of ionic strength and tables of mean activities of the salts involved. Thus, the task of evaluating activities or activity ratios of the solution ions ordinarily does not present a major problem.

The assessment of the correct activities of the adsorbed ions ($a_{Na(ad)}$, $a_{K(ad)}$, $a_{Ca(ad)}$, etc.), on the other hand, constitutes the major problem of the mass law approach and has been the subject of a great amount of theorizing on the part of soil chemists. A few of the more important hypotheses will be presented here.

An early attempt to employ the mass action principle in a study of ion adsorption in soils was made by Kerr (95) in 1928. Kerr assumed that the activities of the solution ions were equal to their concentrations in moles per liter in the soil solution. He assumed further that the activities of the adsorbed cations also were equal to their concentrations in the soil suspension. On this basis his mass law expressions were as follows:

$$Na(ad)/K(ad) \cdot K^+/Na^+ = R_1$$

$$Na(ad)^2/Ca(ad) \cdot Ca^{++}/(Na^+)^2 = k_2$$

$$Na(ad)^3/La(ad) \cdot La^{+++}/(Na^+)^3 = k_3.$$

All quantities are expressed in moles or equivalents per liter of suspension.

Kerr was able to justify experimentally his hypothesis for the cases of certain ion pairs of equal valence (for example, Ca^{++} and Mg^{++}). Subsequent work has shown, however, that although his formulations are usually suitable in the cases of exchanges between ion pairs of equal valence, they are completely inadequate for pairs of unequal valence.

Somewhat later, Gapon (96) attacked the problem of cation exchange in soils using an hypothesis very similar to that of Kerr; that is, he assumed that the active masses of the adsorbed ions were given by their concentrations in the suspension. Thus for ion-pairs of equal valence his equation did not differ essentially from Kerr's, for example, $Na(ad)/K(ad) \cdot K^+/Na^+ = k_1$ where all quantities are expressed as moles or equivalents per liter of suspension. For the case of ion pairs of unequal valence Gapon was led to a rather different formulation. That is, for the $Na^+ - Ca^{++}$ and $Na^+ - La^{+++}$ equilibria:

$$Na(ad)/Ca(ad) \cdot (Ca^{++})^{1/2}/Na^+ = k_2$$

$$Na(ad)/La(ad) \cdot (La^{+++})^{1/3}/Na^+ = k_3.$$

Gapon's expressions for the exchanges between ions of unequal valence are the result of his unconventional writing of the exchange reactions, that is, for example: $Ca_{\frac{1}{2}}X + Na^+ \rightarrow NaX + \frac{1}{2}Ca^{++}$ where X symbolizes the adsorbent. Gapon's equations for the ion pairs of unequal valence have been shown to be entirely unsuitable [see Krishnamoorthy & Overstreet (97)].

It may be appropriate to state at this point that most theories of ion exchange have resulted in essentially the same equation for ion pairs of equal valency. This is uniformly true with theories based on the mass law approach. It is also the case with the kinetic theories. The equations of Jenny and Boyd *et al.* can be shown to be identical with those of Kerr and Gapon for ion pairs of equal valence. The great stumbling block in ion exchange formulations has been the treatment of pairs of unequal valence. The first major advance in this direction was made by Vanselow (51) in 1932.

Vanselow considered carefully the nature of ions in the adsorbed state. He concluded that the ions form complexes with the adsorbent ($Ca\,X_2$, $K\,X$, $Na\,X$, $La\,X_3$, etc.) which exist in the solid phase as components of a solid solution. On this hypothesis, he concluded that the activity of an adsorbed ion should be equal to its mole fraction in the exchange complex. In our present nomenclature Vanselow's hypothesis can be represented as follows:

$$a_{Na(ad)} = Na(ad)/La(ad) + Ca(ad) + Na(ad) + \cdots$$

$$a_{Ca(ad)} = Ca(ad)/La(ad) + Ca(ad) + Na(ad) + \cdots.$$

The quantities $Na(ad)$, $Ca(ad)$, $La(ad)$, etc., are expressed in moles or millimoles per unit volume of suspension and the summation in the denominator includes all of the adsorbed ions. With the foregoing hypothesis concerning the activities of adsorbed ions, Vanselow was led to the following mass law formulations:

$$Na(ad)/K(ad) \cdot a_{K^+}/a_{Na^+} = k_1$$

$$[Na(ad)]^2/\{Ca(ad) \cdot [Ca(ad) + Na(ad) + H(ad) + \cdots]\} \cdot a_{Ca^{++}}/(a_{Na^+})^2 = k_2$$

$$[Na(ad)]^3/\{La(ad)[La(ad) + Na(ad) + H(ad) + \cdots]^2\} \cdot a_{La^{+++}}/(a_{Na^+})^3 = k_3.$$

The quantities $Na(ad)$, $K(ad)$, $Ca(ad)$, etc., are expressed in moles per unit volume of suspension and the activity ratios for the solution ions are evaluated as described previously.

Here again, the expressions for ion pairs of equal valence do not differ essentially from those resulting from other theories. A great divergence, however, will be noted in the expressions for ion pairs of unequal valencies. Vanselow subjected his equations to experimental test with a number of soil colloids and found them to be quite adequate. Moreover, the equations have

been subjected to rigorous test by investigators working with synthetic ion-exchangers (58) and the general applicability of Vanselow's expressions has been established beyond question, at least for monovalent-monovalent, divalent-divalent, and monovalent-divalent ion pairs not involving hydrogen ion.

In the development of his theory, Vanselow assumed that the complexes Na X, K X, Ca X_2, etc., are components of a perfect solid solution in the adsorbent phase. Kielland (98) has proposed certain corrections to Vanselow's formulations that take into consideration the cases of some systems where the complexes (Na X, K X, etc.) presumably do not form perfect solid solutions in the solid phase.

It is important to point out that although Vanselow's solid solution hypothesis has yielded very satisfactory expressions for the equilibrium conditions in an ion-exchange process, this fact does not necessarily constitute evidence that the adsorbed ions actually exist in the solid phase in the form of a solid solution. Indeed, in view of the accumulated information concerning the crystal structure of the clay minerals and the seat of the ion exchange capacity in clays, the solid solution hypothesis seems highly improbable. In the light of modern knowledge a more reasonable and equivalent statement of Vanselow's hypothesis is that the adsorbed cations form a kind of two-dimensional perfect solution on the surfaces of the clay crystals.

A critical examination of the implications of Vanselow's hypothesis has been made recently by Krishnamoorthy & Overstreet (99). These authors conclude that Vanselow's mole fraction theory is perhaps an oversimplification of the problem, since it does not take into consideration the lattice properties of the clay crystal and the fact that the ionic attraction spots must form a definite array on the crystal surfaces. The presence of a fixed array of attraction spots on the adsorbent surface would impose certain restrictions on the adsorption of a polyvalent cation, namely, that the attraction spots involved must be adjacent. These restrictions are not implied in the mole fraction hypothesis.

An analogous problem is encountered in the adsorption of gases on solid surfaces where a single gas molecule can occupy more than one adsorption site. The theory of gas adsorption problems of this type has been worked out by Guggenheim (100).

Krishnamoorthy, Davis & Overstreet (52) have adopted Guggenheim's gas theory for the analogous problem of polyvalent ion adsorption. As a result of this, certain modifications of Vanselow's mole fraction hypothesis for the case of polyvalent ions seems necessary. According to Guggenheim's statistics the active mass of an adsorbed ion A is proportional to $C_A/q_A C_A + q_B C_B + q_C C_C + \ldots$ in which C_A, C_B, etc., are the amounts of the ions present in the adsorbed state expressed in moles or millimoles per unit volume of suspension and q_A, q_B, etc., are parameters dependent on the valency of the ions and the lattice properties of the adsorbent. The statistics deal with a term, Z, which is equal to the number of adsorption sites adjacent to a given

adsorption site. The parameter q mentioned above is defined by the theory in terms of Z and the valency, r, of the adsorbed ion. That is $qZ = rZ - 2r + 2$.

For the cases of clays and synthetic resins it is reasonable to consider the distribution of adsorbed ions as a surface array and assign Z the value 4. On this assumption $q_A = (r_A + 1)/2$. From this it follows that the parameter q has the value 1 for monovalent ions, $1\frac{1}{2}$ for divalent ions, and 2 for trivalent ions. Thus on the basis of the foregoing theory the ion-exchange relationships for the $Na^+ - K^+$, $Na^+ - Ca^{++}$, and $Na^+ - La^{+++}$ pairs become:

$$Na(ad)/K(ad) \cdot a_{K^+}/a_{Na^+} = k_1$$

$$Na(ad)^2/\{Ca(ad)[1\tfrac{1}{2}Ca(ad) + Na(ad) + H(ad) + \cdots]\} \cdot a_{Ca^{++}}/(a_{Na^+})^2 = k_2$$

$$Na(ad)^3/\{La(ad)[2La(ad) + 1\tfrac{1}{2}Ca(ad) + Na(ad) + H(ad) + \cdots]^2\}$$
$$\cdot a_{La^{+++}}/(a_{Na^+})^3 = k_3.$$

The quantities La(ad), Ca(ad), etc., are expressed as moles per unit volume of suspension and the activity ratios of the solution ions are evaluated ordinarily by means of the ionic strength principle.

In an experimental evaluation of cation exchange relationships with soil colloids and synthetic resins, Krishnamoorthy & Overstreet (97) were able to demonstrate that the equations based on Guggenheims' statistics were somewhat superior to Vanselow's formulations, especially in the cases of monovalent-trivalent ion pairs.

Certain other findings of Krishnamoorthy & Overstreet (97) seem worthy of mention at this point, since they give a general picture of the ion exchange problem to date. In the first place, no satisfactory cation exchange relationships could be found for adsorbents which were mixtures of ion-exchange materials of widely different chemical structures, even though good exchange formulations could be found for the individual components of the mixtures. Thus it is possible that many soils may never be amenable to this kind of treatment. Secondly, none of the cation exchange expressions proposed thus far were found suitable for exchanges between ion pairs involving hydrogen ion. This was true for all materials investigated, including clay minerals, soil colloids, and synthetic ion exchangers. Thirdly, although the formulations of Jenny, Boyd, Kerr, Gapon, Vanselow, etc., yielded quite satisfactory and equivalent relationships for ion pairs of equal valence, the equations of Vanselow and those based on Guggenheim's statistics alone were found to be adequate in cases of pairs of unequal valence. This was true with all adsorbents investigated. For monovalent-trivalent ion pairs the statistical equation was unquestionably the better in all systems investigated.

In conclusion, it must be stated that the significance of the chemical state of soils as related to the inorganic nutrition of plants, must be interpreted in terms of soil plant interaction as revealed by the growing plant. Although there are many excellent papers which have not been mentioned

in this review, and although popular chemical subjects such as soil pH and soil analysis for purposes of determining fertility have been scarcely touched upon, it has been the reviewers' intent to select papers to illustrate the prominent features of soil chemistry that are subject to generalization under the broad title of this paper.

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